National Wildlife Disease Program

Fiscal Year 2012 Annual Program Report

The original artwork on this page was created by the National Wildlife Disease Program's Erika Kampe and Sarah Goff

USDA United States Department of Agriculture
Animal and Plant Health Inspection Service

Wildlife Services
NWRC
National Wildlife Research Center

National Wildlife Disease Program

Annual Report: Introduction

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Agency Acronym List

APHIS - Animal Plant Health Inspection Service
ASU - Arizona State University
ARS - Agricultural Research Service
CDC - Centers for Disease Control and Prevention
CSU - Colorado State University
FDA - Food and Drug Administration
FAS - Foreign Agricultural Service
NAHLN - National Animal Health Laboratory Network
NIH - National Institutes of Health
NVSL - National Veterinary Services Laboratories
NWDP - National Wildlife Disease Program
NWHC - National Wildlife Health Center
NWRC - National Wildlife Research Center
SCWDS - Southeastern Cooperative Wildlife Disease Study
USDA - United States Department of Agriculture
USFWS- United States Fish and Wildlife Service
VS - Veterinary Services
WS - Wildlife Services

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National Wildlife Disease Program

Species List

Species List

***Alces alces*: Moose**
***Anas platyrhynchos*: Mallard duck**
***Anser indicus*: Bar-headed Goose**
***Bos primigenius:* Cattle**
***Branta Canadensis*: Canada goose**
***Bucephala clangula:* Common goldeneye**
***Buteo lagopus:* Rough-legged hawk**
***Buteo regalis:* Furruginous hawk**
***Buteo swainsoni:* Swainson's hawk**
***Canis latrans*: Coyote**
***Canis lupus dingo*: Dingos**
***Canis lupus familiaris*: Domestic dog**
***Canis lupus*: Wolves**
***Capra aegagrus hircus*: Goats**
***Castor canadensis:* North American beaver**
***Cervus canadensis*: Elk**
***Chen caerulescens caerulescens*: Lesser snow goose**
***Crocidura leucodon*: White-toothed Shrew**
***Cygnus olor*: Mute Swan**
***Cynomys* spp.: Prairie Dogs**
***Equus ferus caballus*: Horses**
***Felis catus*: Domestic cats**
***Gallus gallus domesticus*: Chickens**
***Herpestes javanicus:* Indian mongoose**
***Lama glama*: Llamas**
***Larus californicus:* California gull**
***Larus delawarensis:* Ring-billed gull**
***Lynx canadensis*: Lynx**
***Lynx rufus*: Bobcat**
***Myocaster coypus*: Nutria**
***Nannospalax nehringi:* Nehring's blind mole rat**
***Neovison vison:* American mink**
***Odocoileus hemionus*: Mule deer**
***Odocoileus virginianus*: White-tailed deer**
***Ondatra zibethicus:* Muskrat**
***Ovis aries*: Sheep**
***Pelecanus erythrorhynchos:* American white pelican**
***Phacochoerus africanus:* Warthog**
***Potamochoerus larvatus:* Bushpig**
***Procyon lotor*: Raccoon**
***Sus scrofa:* Feral swine**
***Tyto alba:* Barn owl**
***Ursus americanus*: Black bear**

National Wildlife Disease Program

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NWDP Surveillance Projects

Classical Swine Fever Negative Cohort

Feral swine are considered an invasive exotic species within the United States. Their populations continue to expand, and currently an estimated 5 million individuals occupy at least 38 states. Feral swine are competent hosts for a number of foreign animal diseases that pose a very real threat to the health and economic viability of the United States livestock industry. Although diseases such as classical swine fever (CSF), Foot-and-Mouth Disease (FMD), and African swine fever (ASF) are not currently found within the United States, feral swine have been identified as a high risk pathway for the transmission of foreign animal diseases into susceptible wildlife and domestic livestock populations. Surveillance for foreign animal diseases in wildlife requires a robust and proactive approach because the majority of individuals within populations of interest ultimately remain unobserved and untested.

Classical swine fever is a highly contagious viral septicemia affecting only swine. Also known as hog cholera, it has been successfully eradicated from many developed nations with extensive swine production, but is still endemic in much of the world. Transmission is typically the result of direct or indirect contact with infectious bodily fluids or consumption of contaminated tissues. Outbreaks in countries free of CSF can have a severe impact on producers due to high swine mortality, the curtailment on exportation of swine and pork products, and from costs incurred to control and eradicate the disease.

The NWDP conducts surveillance in feral swine across the nation. This effort, combined with other surveillance streams in domestic animals and pork products, ensure early detection of CSF if introduced. It also minimizes the risk of transmission to the domestic swine industry, as well as demonstrates freedom from disease for trading partners.

Table 1: Number of feral swine tested for Classical Swine Fever - Fiscal Years 2006-2012

FY2006	FY2007	FY2008	FY2009	FY2010	FY2011	FY2012
21	1138	2098	2111	2560	3156	2739

Figure 1: Feral swine.

The CSF surveillance program was initiated in October 2005 and efforts are aligned with the Federal fiscal year, which begins 1 October and continues through 30 September. Nationally, there is variability in feral swine densities, distributions, WS and state/local/tribal agency control infrastructure, and laws limiting capture methods. As a result, surveillance methodology varies accordingly. The NWDP usually samples

NWDP Surveillance Projects

Classical Swine Fever Negative Cohort, continued

feral swine that are taken for other wildlife damage management purposes by APHIS WS. In some instances, however, the NWDP and other WS personnel will proactively capture and test feral swine for disease. Trapping, snaring, and aerial gunning are the primary methods used to remove feral swine. Blood is collected post-mortem from each pig, centrifuged, and the serum is aliquoted into cryogenic vials that are sent to the APHIS VS Foreign Animal Disease Diagnostic Laboratory on Plum Island, New York for CSF antibody testing. When blood is not available, tonsils are submitted to a NAHLN Laboratory for testing by real-time reverse-transcription polymerase chain reaction. The majority of samples are taken from feral swine populations considered to be at high risk of a foreign animal disease introduction.

The total numbers of samples that have been collected each fiscal year from the inception of the surveillance program are listed in Table 1. In Fiscal Year 2012, 2,748 CSF samples (2,739 serum, 9 tonsil) were collected in 30 states and all were negative for CSF exposure. The numbers of samples collected specifically for CSF testing by state during Fiscal Year 2012 are listed in Table 2.

Table 2: Feral swine tested for Classical Swine Fever, by state, during Fiscal Year 2012.

State	# of samples collected for CSF testing
Alabama	93
Arkansas	137
Arizona	29
California	210
Colorado	3
Florida	315
Georgia	181
Hawaii	189
Illinois	11
Indiana	15
Kansas	85
Kentucky	8
Louisiana	135
Michigan	35
Missouri	114
Mississippi	189
North Carolina	53
New Hampshire	2
New Jersey	1
New Mexico	81
Nevada	2
New York	28
Ohio	30
Oklahoma	226
Oregon	33
South Carolina	79
Tennessee	48
Texas	400
Virginia	13
West Virginia	3

NWDP Surveillance Projects

Pseudorabies

Pseudorabies virus (PRV), also known as Aujeszky's disease, is a viral disease in the family *Herpesviridae* that is an economically important disease in domestic swine. Although it is endemic in most parts of the world, it has been eradicated from commercial swine in the United States. However, feral swine are known reservoirs of the disease and could potentially serve as a proximate or ultimate source for reintroduction into commercial swine. Feral swine populations often overlap with domestic swine operations, which could lead to disease transmission between these groups.

Transmission of PRV occurs primarily through direct animal-to-animal contact. In feral swine, there are no clinical signs of the disease or mortality. Other domestic and wild mammals such as cattle, horses, sheep, goats, dogs, and raccoons can be susceptible, and the disease is often fatal in these species. Humans are not susceptible to PRV.

Table 1: Number of feral swine samples screened for pseudorabies, Fiscal Year 2007-

FY2007	FY2008	FY2009	FY2010	FY2011	FY2012
1255	2564	2449	2563	3161	2894

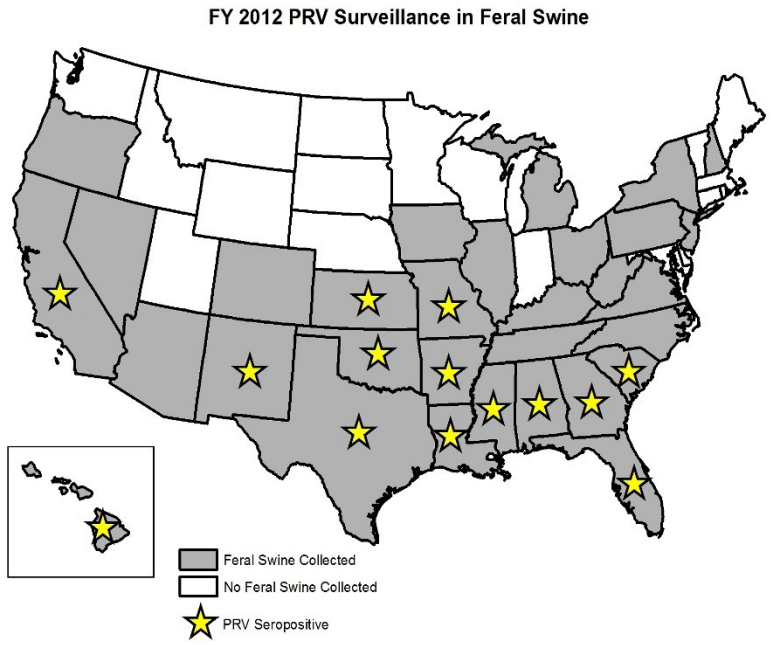


Figure 1: Pseudorabies positive sampling locations from Fiscal Year 2012.

Feral swine are considered an invasive species in the United States and are estimated to cause millions of dollars in damage each year. They are currently known to exist in 38 states with a population estimate of 5 million, but each year they expand into new territory. Wildlife Services' personnel remove about 30,000 feral swine each year for wildlife damage management purposes. The NWDP takes advantage of these removal activities to collect samples for disease surveillance.

The objective of monitoring PRV in feral swine is to establish baseline data and identify trends of prevalence. These data can be used to ensure appropriate levels of biosecurity are implemented on farms in high risk areas, with relatively high levels of PRV circulating in the local feral swine population.

NWDP Surveillance Projects

Pseudorabies, continued

Trapping and aerial hunting are the primary methods used to remove feral swine. Samples are collected from 1 October through 30 September each year. Blood is collected from each pig, centrifuged, and the serum is aliquoted into cryogenic vials for various diseases. Samples are collected whenever possible in areas considered to be high risk, such as landfills or airports, as well as new counties where feral swine have not previously been sampled. Serum samples are collected for PRV testing and for archiving at the NWDP Feral Swine Serum Archive.

In Fiscal Year 2012, samples were shipped to the NWDP office in Fort Collins, Colorado where they were stored in ultra-cold freezers, batched, and shipped to NAHLN laboratories in Washington State and Wisconsin. The samples are tested with the gB enzyme-linked immunosorbent assay.

The feral swine sampling program was initiated in October 2006. The total number of samples that have been collected each fiscal year are listed in Table 1. The number of samples collected for PRV testing during fiscal year 2012 are listed in Table 2, as well as the number of those samples that were positive or suspected positive for PRV exposure during the same period. Figure 1 depicts the states where PRV positive pigs have been collected since the initiation of surveillance in late 2006.

Table 2: Number of pseudorabies samples collected, by state, in Fiscal Year 2012.

State	# of swine sampled for PRV testing	# of positive samples
Alabama	93	8
Arkansas	137	25
Arizona	28	0
California	208	3
Colorado	3	0
Florida	312	103
Georgia	181	13
Hawaii	266	124
Iowa	3	0
Illinois	11	0
Kansas	85	3
Kentucky	8	0
Louisiana	135	16
Michigan	35	0
Missouri	110	1
Mississippi	191	17
North Carolina	53	0
New Hampshire	3	0
New Jersey	1	0
New Mexico	81	3
New York	28	0
Nevada	2	0
Ohio	30	0
Oklahoma	226	65
Oregon	33	0
South Carolina	78	26
Tennessee	48	0
Texas	399	90
Virginia	13	0
West Virginia	3	0

NWDP Surveillance Projects

Swine Brucellosis

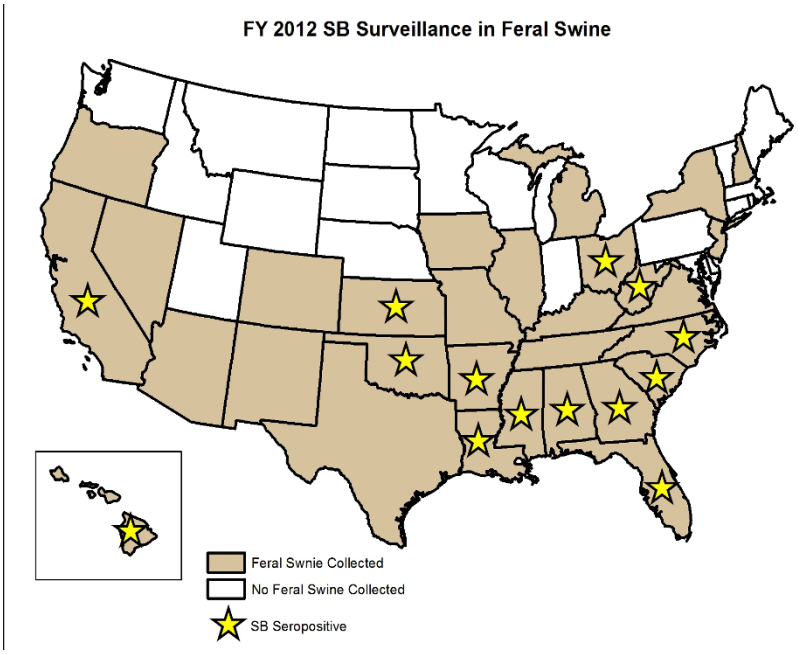


Figure 1: Feral swine

Feral swine are considered an invasive exotic species within the United States. Feral swine carry a number of endemic diseases that can pose a risk to humans, as well as to free-range cattle and domestic swine operations. One such disease is swine brucellosis, caused by the bacterium *Brucella suis*. There are several recognized species of *Brucella*, and each is associated with a specific animal host. While *B. suis* primarily infects pigs, it also can cause disease in cattle, horses, dogs, and humans. Similarly, swine also may become infected with *B. abortus* or *B. melitensis*. The primary route of transmission for *B. suis* in feral swine is thought to be venereal, but vertical transmission via infected milk or oral exposure to infected tissues such as aborted fetuses, and placental tissues also has been documented.

The commercial swine industry in the United States maintains brucellosis-free status in all states, but the presence of brucellosis infected feral swine populations and the potential for them to transmit disease to domestic swine could jeopardize the commercial swine industry. Improved understanding of the prevalence and geographic distribution of brucellosis in feral swine is important for informing and guiding relevant management decisions that will help ensure the security of the United States swine and cattle industries. In addition, feral swine are known to carry other zoonotic *Brucella* species. Brucellosis in humans can manifest as recurrent fever, chills, headaches, and general weakness, and can afflict those infected for extended periods of time. Hunters, wildlife biologists, and anyone involved in butchering or dressing infected feral swine are at risk.

Surveillance is conducted to improve our understanding of the apparent prevalence and geographic distribution of brucellosis in feral swine. Such knowledge allows us to increase our ability to identify areas of increased risk for re-introduction of brucellosis into domestic swine and cattle populations, as well as areas of higher risk for hunters and others who have contact with feral swine.

Table 1: Number of samples screened for swine brucellosis, Fiscal Years 2006-2012

FY2006	FY2007	FY2008	FY2009	FY2010	FY2011	FY2012
2	1240	2474	2730	2540	3155	2645

NWDP Surveillance Projects

Swine Brucellosis, continued

Trapping, snaring, and aerial gunning are the primary methods used to remove feral swine. Blood is collected post-mortem from each pig, centrifuged, and the serum is aliquoted into cryogenic vials that are shipped to the NWDP in Fort Collins, Colorado. Samples are then batch shipped for diagnostic testing using the fluorescence polarization assay (FPA). From October 2011 to July 2012, testing was done at the Kansas State-Federal Brucellosis Laboratory, Topeka, KS. Since July 2012, testing has been done at the Kentucky Veterinary Services Laboratory in Frankfort, KY. Confirmatory testing of presumptive positive samples is done at the NVSL in Ames, IA.

Table 2: Number of samples screened for swine brucellosis by state, and number of positive samples, in Fiscal Year 2012.

State	# of swine tested for SB	# positive samples
Alabama	91	19
Arkansas	124	20
Arizona	29	0
California	208	1
Colorado	3	0
Florida	243	36
Georgia	160	5
Hawaii	264	41
Iowa	3	0
Illinois	10	0
Kansas	84	4
Kentucky	8	0
Louisiana	131	2
Michigan	34	0
Missouri	74	0
Mississippi	190	1
North Carolina	52	12
New Hampshire	3	0
New Jersey	1	0
New Mexico	81	0
Nevada	2	0
New York	28	0
Ohio	29	1
Oklahoma	223	9
Oregon	33	0
South Carolina	79	11
Tennessee	48	0
Texas	396	0
Virginia	11	0
West Virginia	3	1

NWDP Surveillance Projects

Swine Influenza

Swine play a unique role in the epidemiology of influenza A viruses. They have similar cellular receptors to both birds and humans and, consequently, can become infected with subtypes of influenza A viruses associated with each group. If different influenza A subtypes are present within an individual, there is an opportunity for genetic reassortment to occur between subtypes. This sort of mutation, known as antigenic shift, causes a sudden and significant change in the genetic make-up of a virus, which may lead to the creation of a potentially more infectious subtype. Swine influenza virus (SIV) has received increasing attention since the emergence in March, 2009 of the novel pH1N1 subtype (an influenza subtype that affects humans, domestic swine and several other species). H1N1 was the dominant influenza subtype in the United States domestic swine populations since its discovery in the early 1930s; however, a new subtype (H3N2) created by a triple reassortment of avian, human, and porcine genes was discovered in 1998 in commercial swine populations. This is now the dominant subtype across the country. The 2009 novel pH1N1 is similar to H3N2 in that it contains an assortment of genes from humans, birds, and pigs. This subtype also contains genes of Eurasian swine origin that had not previously been detected in North American swine.

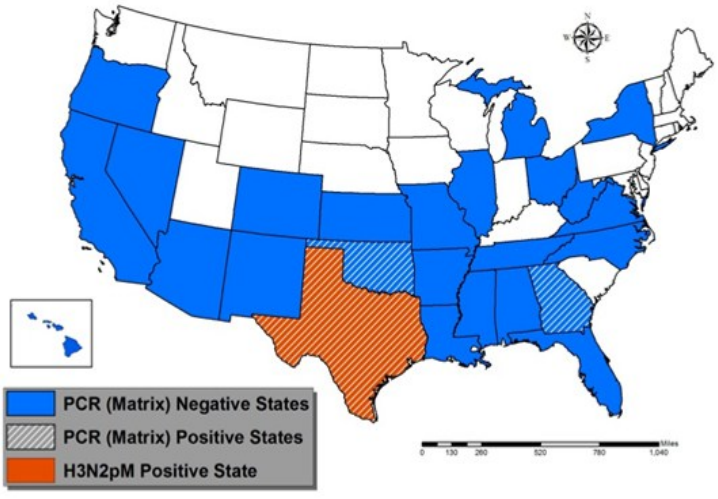
Nasal Swab Samples (Matrix)			
State	Negative	Positive	Total
AL	76		76
AR	114		114
AZ	29		29
CA	23		23
CO	3		3
FL	279		279
GA	143	6	149
HI	100		100
IL	1		1
KS	79		79
LA	124		124
MI	28		28
MO	52		52
MS	143		143
NC	53		53
NM	64		64
NV	2		2
NY	25		25
OH	24		24
OK	224	2	226
OR	17		17
TN	32		32
TX	334	1	335
VA	2		2
WV	3		3
Total	**1974**	**9**	**1983**

Table 1: Sample results from SIV nasal swab surveillance

Feral swine pose a risk of introducing or reintroducing endemic and foreign animal diseases to American livestock and wildlife populations. Feral swine can also serve as a reservoir for zoonotic diseases that are a great concern to human health. Wildlife Services is working with several federal agencies to establish surveillance programs that will detect influenza viruses, which pose a threat to domestic livestock and human health.

Wildlife Services has cooperative agreements with various landowners and agencies to remove feral swine that are causing damage to agriculture and natural

Figure 1: Results from SIV nasal swab surveillance.



NWDP Surveillance Projects

Swine Influenza, continued

Table 2: Sample results for SIV antibody surveillance.

Serum Samples (ELISA)			
State	Negative	Positive	Total
AL	73	10	83
AR	77		77
AZ	28	1	29
CA	169	7	176
CO	3		3
FL	206	12	218
GA	99	6	105
HI	192	7	199
IL	10		10
IN	6		6
KS	55	7	62
KY	1		1
LA	90	3	93
MI	22		22
MO	23	1	24
MS	143	3	146
NC	10	7	17
NH	2		2
NJ	1		1
NM	67	2	69
NV	2		2
NY	26	1	27
OH	22	2	24
OK	123	4	127
OR	3		3
SC	63	2	65
TN	45	2	47
TX	235	104	339
VA	8	1	9
WV	3		3
Total	**1807**	**182**	**1989**

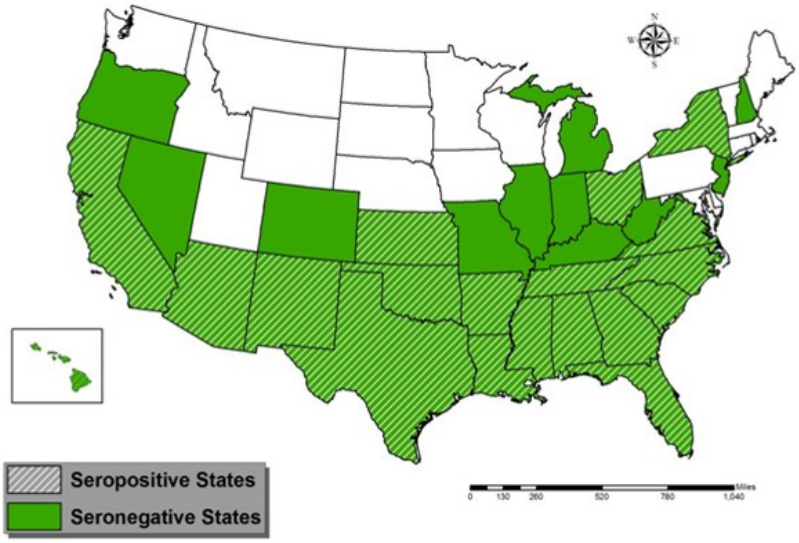


Figure 2: Results from SIV antibody surveillance in feral swine.

resources. When feral swine are euthanized, the NWDP opportunistically collects samples for disease surveillance. A paired nasal swab and serum sample are collected from each animal. Nasal swabs are placed into brain heart infusion media, refrigerated, and shipped to a predetermined NAHLN laboratory for influenza testing using real-time reverse-transcription polymerase chain reaction. All positive samples are further tested for the N1 neuraminidase protein and with virus isolation. If virus isolation is positive, the sample is sequenced. Additionally, blood serum samples are aliquoted into cryogenic vials and shipped to the NWDP where they are tested for antibodies to influenza A viruses using the IDEXX b -enzyme linked immunosorbent assay test.

The NWDP began surveillance for SIV on 1 November, 2010. Sampling has been conducted in 30 of the 38 states that have feral swine. Nasal swabs have been collected in targeted counties and serum samples continue to be collected from all other feral swine throughout the United States. As of 30 September, 2012, NWDP has sampled and submitted 1,983 nasal swabs and 1,989 serum samples. The NAHLN Laboratories have tested all 1,983 nasal swabs and 9 tested matrix positive by PCR. Further testing of the matrix PCR positive samples resulted in one sample sequenced to the pandemic variant H3N2pM. Of the 1,989 serum samples, 182 (9.1%) were positive for Type A influenza exposure.

NWDP Surveillance Projects

Trichinella

Nematodes (roundworms) of the genus *Trichinella* are parasites of carnivorous and omnivorous animals. Transmission is through ingestion of muscle tissue containing encysted larval worms. When ingested, the larval worms leave the cyst and quickly mature into adults in the host small intestine. Adult parasites produce larvae, which migrate through the bloodstream to striated muscle tissue where they form cysts, completing the life cycle. Trichinellosis is the disease caused by *Trichinella* organisms. Symptoms include nausea, diarrhea, vomiting, fatigue and fever, followed by aching joints, muscle pain, rashes, itchy skin and weakness. If a high worm burden develops, heart function and breathing may be affected, and can result in death. Even in mild cases, weakness, fatigue and diarrhea may persist for months. In the past, most human cases could be traced to pork. Domestic swine were commonly fed raw-meat garbage, and pork products were not always cooked adequately. Prior to World War II, an estimated 36% of people in the United States had contracted trichinellosis. The prohibition of feeding garbage to swine and education about the need to cook pork thoroughly has greatly decreased the incidence of trichinellosis in the United States. Today most cases are associated with eating raw or undercooked wild game meats. Only 72 cases were reported between 1997 and 2001.

Figure 1: *Trichinella spiralis* larvae.

Trichinellosis is most commonly caused by the species *Trichinella spiralis*, which is especially well adapted to domestic swine and has a cosmopolitan distribution. There are at least three other endemic species of *Trichinella* found in wildlife in North America. Recently developed molecular biologic assays for larvae allow specific genotypes of *Trichinella* to be identified. It has been hypothesized that prior infection with *Trichinella* species effectively provides cross-immunity to swine from infection by the entire genus, and further, that *T. spiralis* will not be maintained in feral swine and other scavenging animals without the presence of infected domestic swine. The dynamics of parasite exchange between domestic and feral swine is not well understood. With increasing demand for "organic" pork there is an increase in "pasture pig" operations in the United States. By regulatory definition, organic pork comes from domestic pigs that are allowed access to pasture at least once per day. Thus, the opportunities for interactions between domestic and feral swine are increasing.

Since 2009, the NWDP has been collecting 2,000 to 3,000 serum samples per year from feral swine to aid in identifying areas of potential risk for the domestic swine industry. Samples from FY 2009 to 2011 were submitted to the ARS Laboratory in Bethesda, Maryland where they were tested by enzyme-linked immunosorbent assay for the presence of antibodies to *Trichinella* species. Results indicate that feral swine

NWDP Surveillance Projects

Trichinella, continued

in 23 states have been exposed to *Trichinella.* Figure 1 shows state with Trichinella positive serum samples collected in Fiscal Year 2011 and analyzed in Fiscal Year 2012. Beginning with the Fiscal Year 2012 serum samples testing for *Trichnella* exposure is being done by NWDP staff and CSU. Results are pending. Although widespread, the apparent prevalence rate is fairly low, about 2%. The data also suggest clustering of positive cases. That is, when one infected swine is found others in the same sounder (family group) or the same general area tend to be infected as well.

In Fiscal Year 2011, the NWDP began collecting paired serum and tongue samples from feral swine in selected states and counties where previous sampling indicated exposure to *Trichinella*. The tongue, specifically the base of the tongue, is the preferred sample because *Trichinella* larvae display a predilection for this tissue. The larvae are being extracted and genotyped to species by the ARS. Serum from tissue positive *Trichinella* individuals will be examined to determine if species-specific markers are present in blood. In Fiscal Year 2012 disease biologists in six states collected and submitted tongue samples. Genotyping is proceeding at the ARS laboratory and the results are pending.

This work will identify the species of *Trichinella* carried by feral swine as well as their immune status. Results will be useful in developing immunologically-based strategies for protecting pasture-raised pigs from infection.

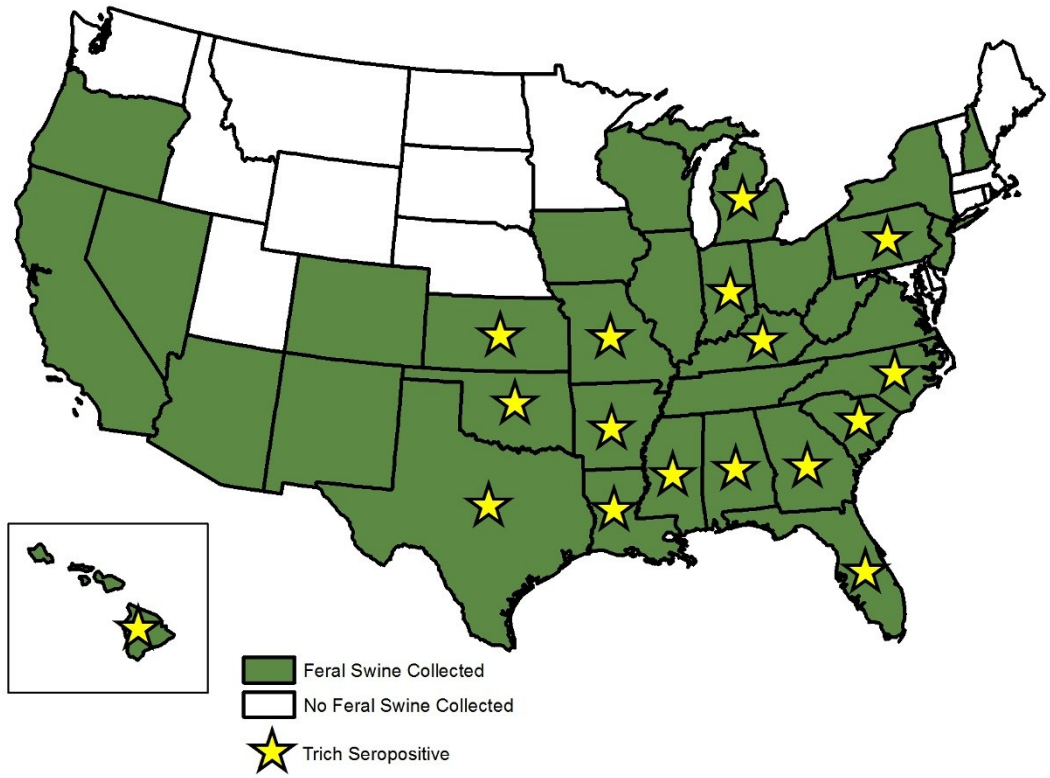


Figure 1: Results from *Trichinella* spp. surveillance in Fiscal Year 2011.

NWDP Surveillance Projects

Hepatitis E Virus

Hepatitis E Virus (HEV) is one of the five hepatitis viruses (A, B, C, D, and E) that can cause inflammation of the liver in humans. HEV is a single-stranded RNA virus in the genus *Hepevirus*. This disease is prevalent in most developing countries and is primarily spread through contaminated water supplies or consumption of undercooked meat. Since HEV is considered a waterborne disease, major outbreaks in humans are typically observed immediately following typhoons and heavy rains that result in flooding. There are four genotypes of HEV found throughout the world. Genotypes 3 and 4 are zoonotic, with domestic swine and several wildlife species (rodents, deer, feral swine) potentially serving as reservoirs. Recent studies have indicated that individual domestic swine operations may have infection rates as high as 95%.

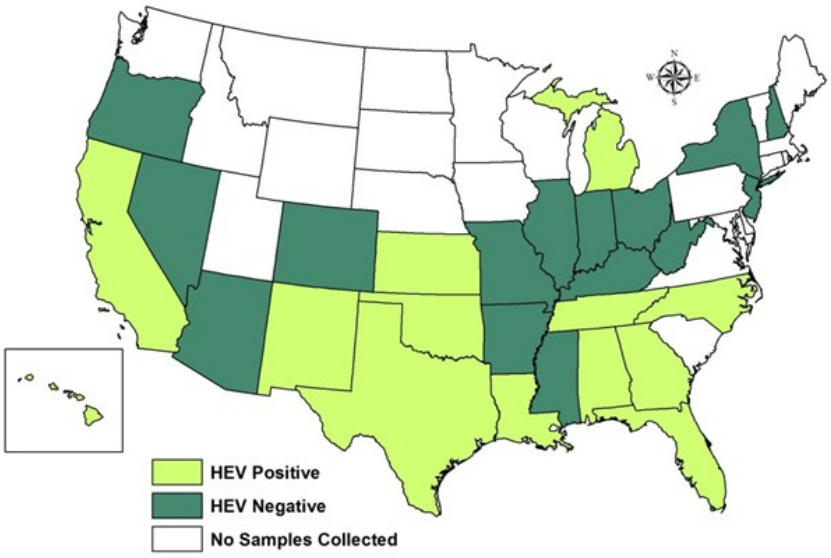


Figure 1: Distribution of HEV feral swine samples collected in Fiscal Year 2012.

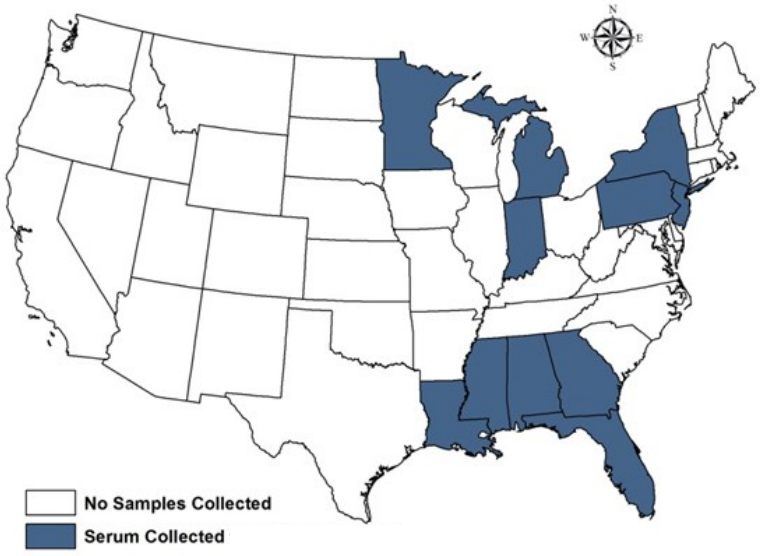


Figure 2: Distribution of HEV deer samples collected in Fiscal Year 2012.

The NWDP is collaborating with the NIH and the FDA to determine whether HEV is circulating in wildlife species and, if so, which genotypes are most prevalent. Surveillance for HEV will allow for both detection and identification of the genotypes circulating in feral swine and cervid populations. Paired samples (serum and fecal) are being collected to distinguish active shedding from exposure (seroprevalence).

Wildlife disease biologists are collecting blood samples from feral swine, cervids, and mongoose that are removed due to wildlife damage management practices. Whole blood is collected and centrifuged to harvest the serum, which is stored in 2 ml cryogenic vials and

Hepatitis E Virus, continued

shipped to the NWDP office in Fort Collins, Colorado. Staff biologists prepare samples from the field and batch-ship them monthly to the NIH laboratory for testing.

The NWDP is in its second year of HEV surveillance in wildlife. A total of 3,102 samples were collected from 30 states during Fiscal Year 2012 (1 October, 2011 to 30 September, 2012). Of the 2,497 feral swine samples tested at the NIH laboratory, 128 tested positive for exposure to HEV (Figure 1). Of the 828 cervid samples collected from 11 states, only 598 were tested due to funding and low prevalence in cervid populations (Figure 2). All of the cervid samples were negative. NIH is currently working to validate an enzyme-linked immunosorbent assay for Indian mongoose samples.

Table 1: Number of serum samples collected, by state, during Fiscal Year 2012.

State	Feral Swine	Cervid	Indian Mongoose
Alabama	93	27	
Arizona	29		
Arkansas	137		
California	173		
Colorado	3		
Florida	304	20	
Georgia	181	91	
Hawaii	260		7
Illinois	11		
Indiana	6	16	
Kansas	84		
Kentucky	4		
Louisiana	125	9	
Michigan	34	28	
Minnesota		11	
Mississippi	190	20	
Missouri	57		
Nevada	2		
New Hampshire	3		
New Jersey	1	172	
New Mexico	81		
New York	28	8	
North Carolina	52		
Ohio	30		
Oklahoma	221		
Oregon	30		
Pennsylvania		196	
Tennessee	48		
Texas	307		
West Virginia	3		
Total	**2497**	**598**	**7**

NWDP Surveillance Projects

Bluetongue Virus and Epizootic Hemorrhagic Disease Virus

Figure 1: Bluetongue virus and Epizootic Hemorrhagic Disease virus can cause mortality in livestock.

Bluetongue (BTV) and Epizootic Hemorrhagic Disease (EHDV) are devastating diseases of ruminants, including deer, sheep, and other livestock. It is estimated that BTV costs the United States' cattle and sheep industries $125 million annually and $3 billion worldwide. EHDV can have high mortality rates that can reduce local deer populations. These viruses are transmitted by several species of *Culicoides*, also known as biting midges or no-see-ums. Both viruses are found in the genus Orbivirus with numerous subtypes identified throughout the world. In the United States, there are six serotypes of BTV (1, 2, 10, 11, 13, and 17) and two serotypes of EHDV (1 and 2). Recent outbreaks of BTV-8 in Europe have prompted concerns that these foreign strains could enter the United States and have devastating impacts on livestock and wildlife.

The NWDP is collaborating with the ARS to identify hot spots where EHDV/BTV outbreaks are occurring and establish surveillance around these locations. One objective is to identify the distribution/diversity of *Culicoides* species in these areas. A second objective is to test for EHDV/BTV virus in Culicoides samples, and ultimately identify which species are vectors for the virus in each region.

Wildlife disease biologists identified trapping locations and set out CDC light traps in the evening and left them overnight to capture insects. The CDC light traps have an ultraviolet black light, as well as a canister of dry ice to emit CO^2 throughout the night. Insects were removed from the traps in the morning and immediately placed on dry ice. All insects were shipped to the NWDP office weekly during the collection period, where they were sorted and identified. After identification, insects were tested by the ARS laboratory in Manhattan, Kansas using a multiplex real-time reverse-transcription polymerase chain reaction that can simultaneously screen for multiple serotypes of both BTV and EHDV.

In FY2012, EHDV/BTV surveillance was conducted in 6 states that had reported deer die-offs due to EHDV. CDC light traps were set up near these locations to trap *Culicoides* that will be identified and tested later this year. A total of 132 trap nights were conducted during Fiscal Year 2012 (Table 1). Surveillance for Fiscal Year 2013 will expand to approximately 10 states due to more states reporting deer die-offs in EHDV.

Table 1: Trap nights by state for Fiscal Year 2012.

State	Number of trap nights
Maine	30
Maryland	28
Montana	24
New Jersey	33
New York	10
North Dakota	7
Total	132

NWDP Surveillance Projects

Toxoplasma gondii

Toxoplasmosis can be a serious disease in humans, affecting the developing fetus of women who acquire infection during pregnancy, and individuals who are immuno-compromised as a result of HIV-1 infection, lymphoma or immunosuppressive therapy. The causative agent is *Toxoplasma gondii,* a protozoan parasite with worldwide distribution. Humans can become infected with *T. gondii* by consuming undercooked meat carrying tissue cysts, or accidental ingestion of sporulated eggs (oocysts) from the environment, which are shed in the feces of felids (the cat family, Felidae). Currently about 12% of the United States population is thought to be infected with *T. gondii* and approximately 20% of deaths attributed to food-borne pathogens are due to *T. gondii* infection. Infection in healthy adults is controlled by the immune system, and rarely causes disease.

The incidence of *T. gondii* in enclosure-reared livestock, including swine, in the United States is low due to improved animal husbandry practices. However, *T. gondii* infection can be more common in free-ranging and backyard livestock. Most healthy livestock are resistant to clinical toxoplasmosis, but infections during pregnancy can cause abortions. Infection with *T. gondii* in wildlife is common, and in some species infection rates can be quite high (e.g., white-tailed deer, bear, feral swine, raccoons and bobcats), though they remain asymptomatic. *T. gondii* infection in wildlife species can be a source of infection for humans and livestock, and can be an indicator that *T. gondii* oocysts are present in the local environment.

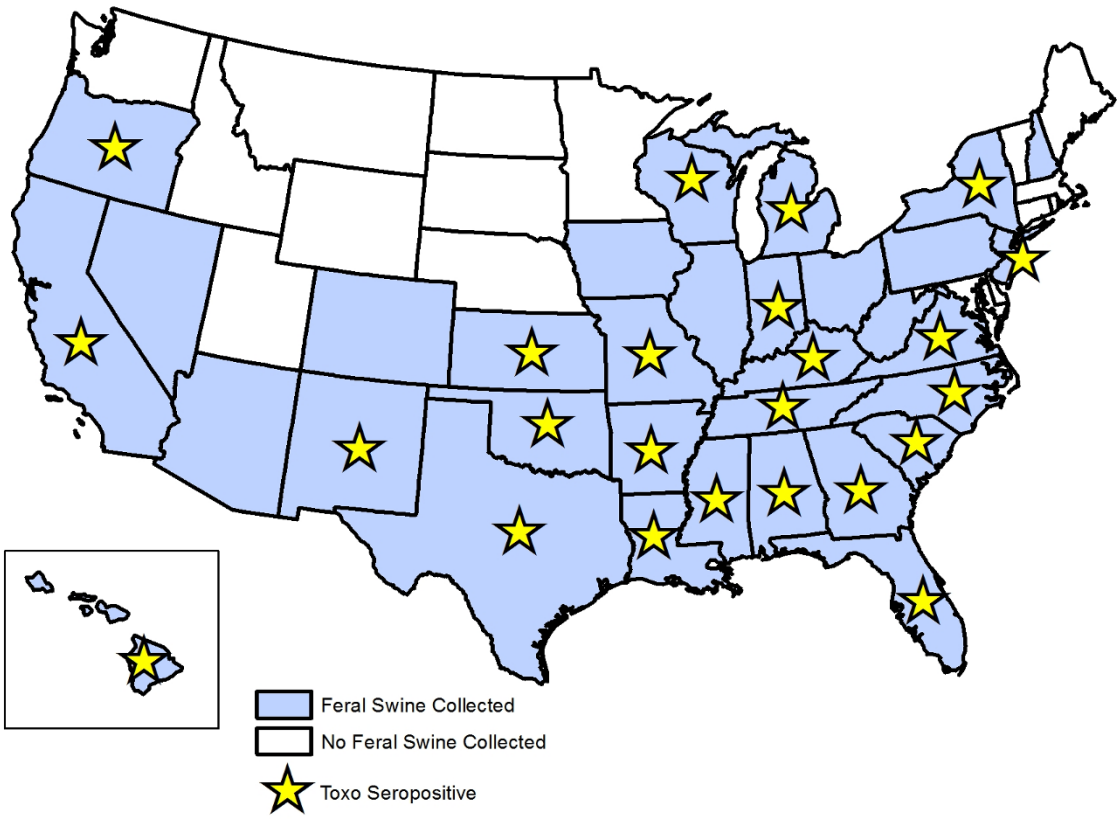


Figure 1: States with feral swine samples tested for *Toxoplasma gondii.*

NWDP Surveillance Projects

Toxoplasma gondii, continued

The NWDP is collaborating with a variety of state and federal cooperators to learn more about the distribution, transmission and prevalence of *T. gondii* in wildlife. Researchers at the Animal Parasitic Diseases Laboratory of the ARS in Beltsville, Maryland, with expertise in laboratory diagnostics and the epidemiology of toxoplasmosis, are key cooperators in the wildlife testing efforts, which support the broader goal of improving food safety in the United States.

The primary objective is to survey feral swine and other species in the United States to determine the distribution and apparent prevalence of *T. gondii*. When combined with habitat suitability models, the results may help predict the risk of future spread of *T. gondii* to new areas. A second objective is to determine the genotype of *T. gondii* in selected areas. Lastly, ARS hopes to find biomarkers in serum that correlate with genotypes of *T. gondii*, which would increase the utility of sero-surveillance.

Figure 2: Group of feral swine.

At the national level, serology for *T. gondii* antibodies is performed with serum from feral swine sampled in 36 states. From Fiscal Year 2009-2011 serum samples were sent to ARS. Fiscal Year 2011 is the most recent year for which results are complete. The collaboration has enabled spatial analysis of the distribution of *T. gondii* in feral swine. Based on these distribution maps, WS biologists in six states are now collecting selected tissues from feral swine in focal counties along with serum samples. *T. gondii* are extracted from swine tongue tissue and used to inoculate mice. The mice are sacrificed after 10 days and assayed for *T. gondii*, which is then be isolated for genotyping. The tissue collections will allow ARS scientists to isolate and identify different genotypes of *T. gondii.* This information will increase our understanding of the strains of *T. gondii* circulating in feral swine, and potential spillover between feral swine and pasture-raised, domestic swine.

Fiscal Year 2012 serum samples are being tested for *T. gondii* exposure at CSU. Results from the tissue extraction study at ARS, as well as serology results, are pending.

The ARS also conducted diagnostic tests on wildlife samples WS collected from Alaska and Colorado since 2009. In Alaska, a new atypical mouse-virulent genotype was isolated from black bear samples collected near Alexander Lake in 2009. Such atypical genotypes in general are thought to be associated with cases of active toxoplasmosis in immunocompetent persons who are resistant to typical genotypes. Initial results in Colorado include the isolation of *T. gondii* from four bird species not known to be hosts prior to this study: barn owl, ferruginous hawk, rough-legged hawk, and Swainson's hawk.

NWDP Surveillance Projects

Baylisascaris

The disease producing capability of nematodes in the genus *Baylisascaris* have become an increasing health concern during recent years. The main species of concern is *Baylisascaris procyonis*, a large intestinal nematode commonly found in raccoons throughout the United States. *B. procyonis* is the most commonly recognized cause of clinical larva migrans in both animals and humans and is best known as a cause of fatal or severe neurologic disease. Over 90 species of birds and mammals are susceptible to *B. procyonis* infections resulting in high morbidity and/or mortality rates. Highly susceptible intermediate hosts, include various rodents, lagomorphs, and avian species. Intermediate hosts frequently become infected by foraging among communal raccoon latrine sites, which provide granivorous birds and mammals a readily available cache of undigested seeds.

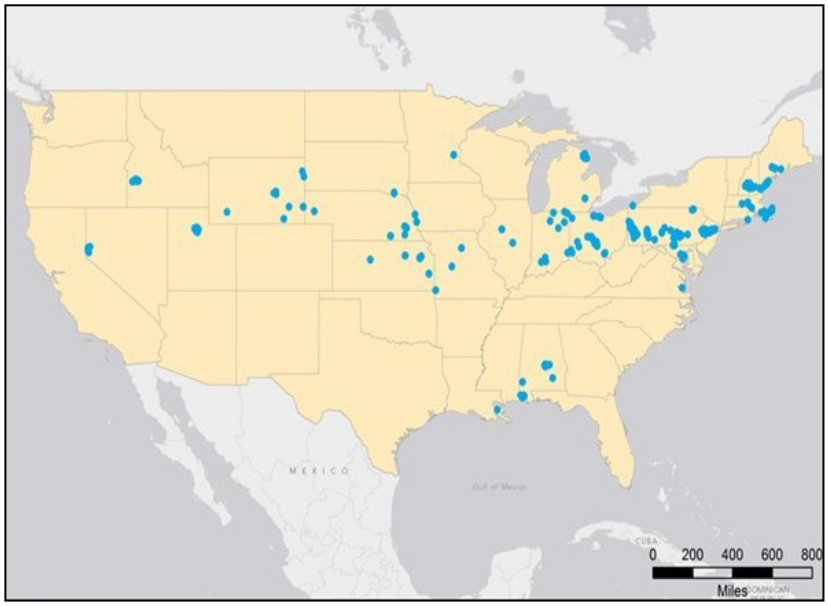


Figure 1: Distribution of the *Baylisascaris* species samples submitted from June 2011 to June 2012. (Note: Samples submitted from Florida are not represented on this map.)

During the last decade *B. procyonis*, commonly referred to as raccoon roundworm, has become recognized as a cause of a severe debilitating disease, Baylisascariasis, in humans and in multiple animal species. The definitive host, raccoons (*Procyon lotor*) are pervasive throughout rural, suburban, and urban settings. Raccoons readily adapt to living in peridomestic settings; exploiting opportunistic food sources (gardens, compost bins, bird feeders, garbage, and pet food), building dens in attics, crawl spaces, under porches, and benefitting from a lack of predation and hunting pressures.

Baylisascaris procyonis is geographically distributed across the United States with prevalence estimates ranging from 10%-82%. Local prevalence rates can vary over time due to natural migration and translocation of animals (either accidental or intentional). Thus, it is imperative that public health and school officials, physicians, wildlife professionals, and veterinarians are aware of the potential health risks *B. procyonis* can pose to both humans and animals.

The NWDP initiated a *Baylisascaris* surveillance project in June of 2011 in order to determine the apparent prevalence and distribution of *Baylisascaris* species in raccoon and skunk populations at a national scale; to identify and document factors associated with *B. procyonis* expansion; correlate human cases with infection rates in wildlife and domestic animals; and compile risk assessment maps for humans living in endemic areas. The *Baylisascaris* surveillance project ended July 13th, 2012. NWDP biologists and cooperators have submitted over 1,300 samples collected in twenty-three states.

NWDP Surveillance Projects

Plague

Yersinia pestis is a flea-borne bacterium and the agent responsible for plague. The pathogen is traditionally described as cycling through small mammal populations, with an enzootic cycle and an epizootic cycle. The enzootic cycle of plague is maintained among rodent hosts and their fleas; however, transmission to humans and other mammals can occur through flea bite or direct contact and, in some cases, results in severe morbidity and mortality.

A majority of human plague cases in the United States are associated with peridomestic transmission in non-urban areas, often involving bites from rodent fleas or even pneumonic transmission from contact with domestic pets. From 1950 through 2009, 464 plague cases were reported in the United States. Despite this relatively limited occurrence in humans, evidence of plague exposure in regions of the Western United States in non-domestic rodents and carnivores is substantial. This is likely because plague is essentially a disease of rodents, many of which survive plague exposure or have limited to no clinical symptoms at all. Carnivores that prey upon these rodents, or are exposed to their fleas, can also be exposed to the bacterium.

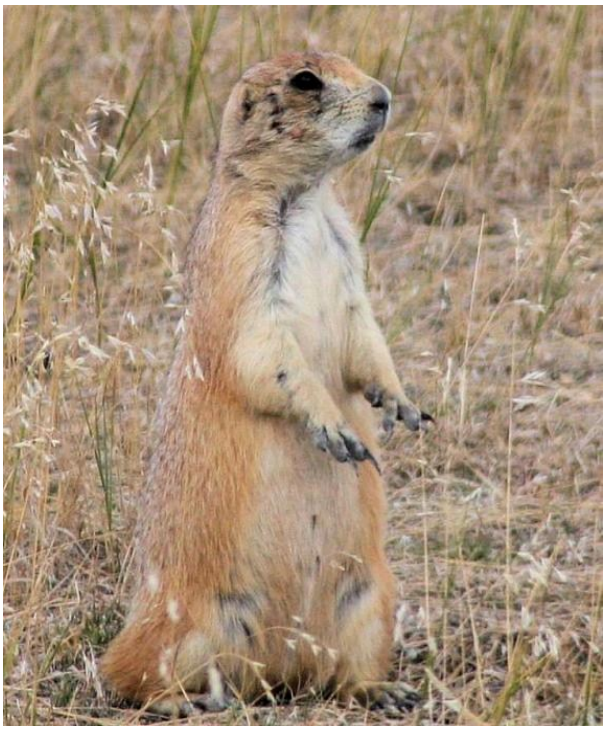


Figure 1: Plague can cause significant mortality in prairie dogs.

Like other areas in the world, plague activity in the United States is often difficult to detect for extended periods of time. There is typically limited evidence of regular plague transmission, but these low activity periods are interspersed by occasional epizootics that result in highly visible die-offs of some rodent species. Prairie dogs suffer the most dramatic die-offs in the United States, with plague affected populations suffering up to 98% mortality. While documenting these die-offs is an efficient and low-cost way to monitor plague dynamics, they often are detected only after an epizootic has been underway and may not necessarily serve as an early warning system.

Monitoring plague exposure, or seroprevalence, through active surveillance of other animals, such as coyotes that can act as sentinel species, is a viable option for monitoring plague dynamics. The objective for this surveillance project is to determine plague exposure in wildlife, on a national scale, to better understand transmission dynamics and risk of human exposure.

The NWDP has directed a long-term plague surveillance program, in cooperation with the CDC, the Washington State Health Department, the Texas Department of Health, and other local and tribal agencies in the United States. *Yersinia pestis* has not colonized the eastern half of the country since its introduction to the United States at the beginning of the 20th century, and surveillance efforts are primarily restricted to areas west of the 100th meridian. Plague surveillance by the NWDP is conducted through opportunistic sampling of wildlife species, with a focus on sentinel species, such as coyotes. Blood samples are collected

Plague, continued

on Nobuto strips by wildlife disease biologists and other WS personnel and disseminated to the CDC for screening. Samples have been collected from multiple species and across large spatial scales since 2005, although testing levels were reduced by the CDC in 2011.

To date, 53,295 samples have been collected from 2005-2012 (Figure 1) as part of the *Y. pestis* and *Francisella tularensis* surveillance program. Subsets of these samples (n=30,020) were chosen for plague screening based on geographic location, and included samples collected from multiple species across 29 states (Table 1, Figure 2). Overall there was substantial exposure to *Y. pestis* in wildlife in the western United States, with the highest apparent seroprevalence rates in coyotes (mean=10%). For Fiscal Year 2012, 79 samples were screened.

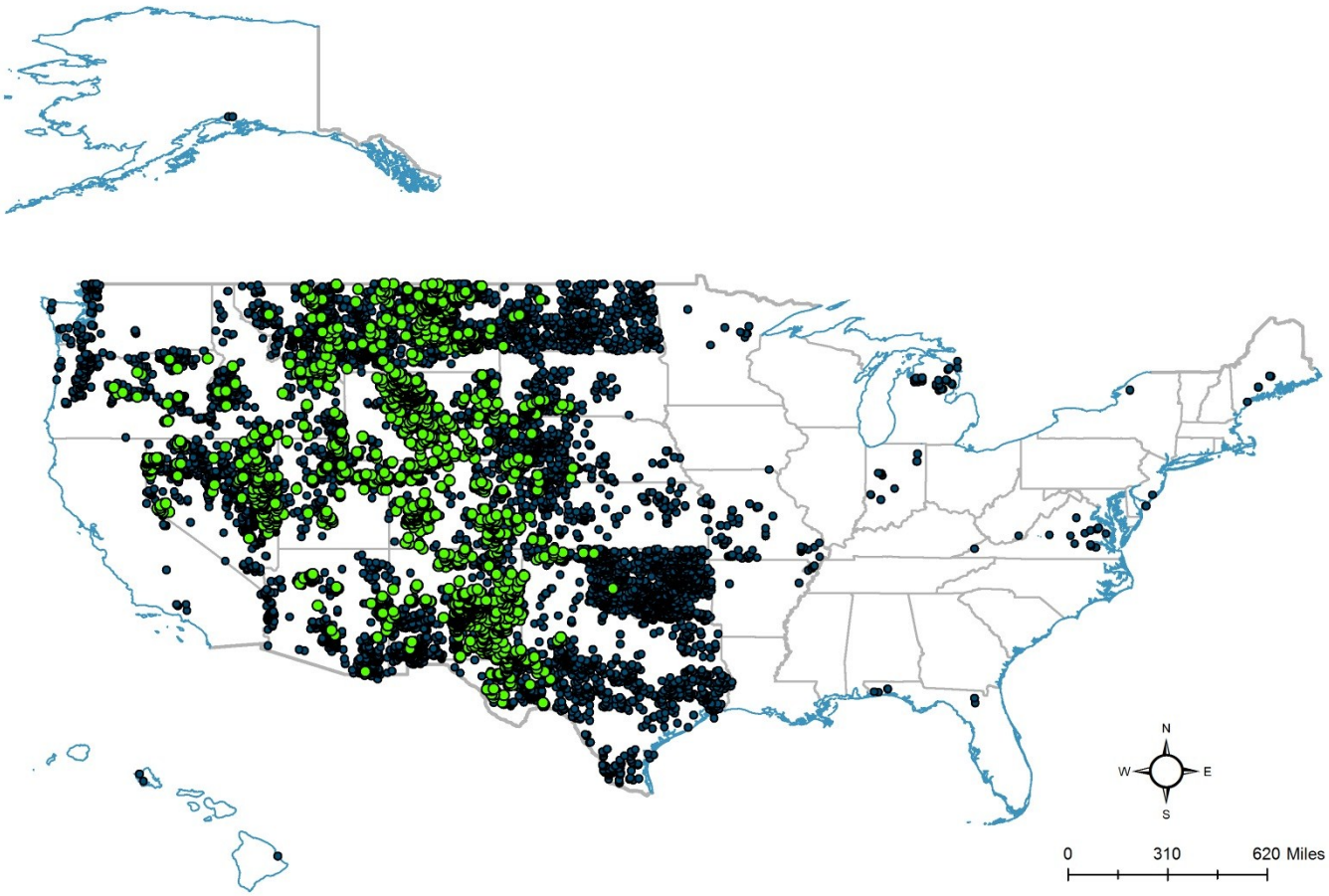


Figure 2: Plague surveillance in wildlife, 2005-2012. Blue symbols represent a *Y. pestis* negative sample; green symbols represent a *Y. pestis* positive sample.

NWDP Surveillance Projects

Tularemia

Tularemia is a highly infectious zoonotic disease caused by the bacterium *Francisella tularensis*. First described in the early 1900's and initially isolated in Tulare County, California, *F. tularensis* has now been separated into four subspecies based on geographic occurrence and ecological associations; however *F. tularensis tularensis* (Type A) and *F. tularensis holarctica* (Type B) are the subspecies most often associated with human disease. Both are found in the United States, but have different geographic distributions. Tularemia is considered to be one of the most infectious pathogens ever studied; its ability to readily aerosolize and potential use as a bioterrorism agent make it part of the National Notifiable Diseases Surveillance System.

Figure 1: Rabbit die-offs can be associated with tularemia.

More than 200 species have been documented with naturally occurring *F. tularensis* infections and multiple vectors, including ticks and tabanid flies, are thought to be involved in transmission; however, only a few vectors and host species are thought to play an important role in perpetuating the pathogen. *Francisella tularensis tularensis* typically cycles in terrestrial environments and is primarily associated with lagomorphs and arthropod vectors. Flies and mosquitoes are thought to be mechanical vectors, while ticks are biological vectors and may also potentially act as a reservoir. *Francisella tularensis holarctica* is associated with aquatic environments, particularly rodent species. Approximately 100-150 human tularemia cases occur every year in the United States and most are attributed to tick bites, although outbreaks linked to aerosolized tularemia, biting-flies, and mosquitoes, have been documented as well. Human infections can result in severe clinical symptoms, including fever, localized ulcer at cutaneous inoculation site, and respiratory distress if exposed through inhalation, although infections are rarely fatal when treated with antibiotics.

By monitoring tularemia exposure, or seroprevalence, in wildlife we can better understand the ecological dynamics governing tularemia in the United States. The NWDP, in conjunction with animal damage management activities or other research projects conducted by WS, opportunistically obtains samples from multiple species from across the country to screen for tularemia exposure. Samples are collected on Nobuto blood filter strips, in cooperation with state and other federal agencies, and screened at the CDC using standard protocols.

NWDP Surveillance Projects

Tularemia, continued

To date, 53,212 samples have been collected from 2005-2011 (Figure 1) as part of the *Y. pestis* and *Francisella tularensis* surveillance program. Subsets of these samples (n=21,719) were chosen for tularemia screening based on geographic location, and included samples collected from multiple species across 46 states (Table 1, Figure 1). Tularemia exposure is very low overall, although when it was identified, it was widely spread across states and species. Illinois (mean apparent seroprevalence = 4.54) and Missouri (mean apparent seroprevalence = 2.57) had the highest occurrence, relative to other states; however, positive sample sizes were still very low. In Fiscal Year 2012, 68 samples were screened.

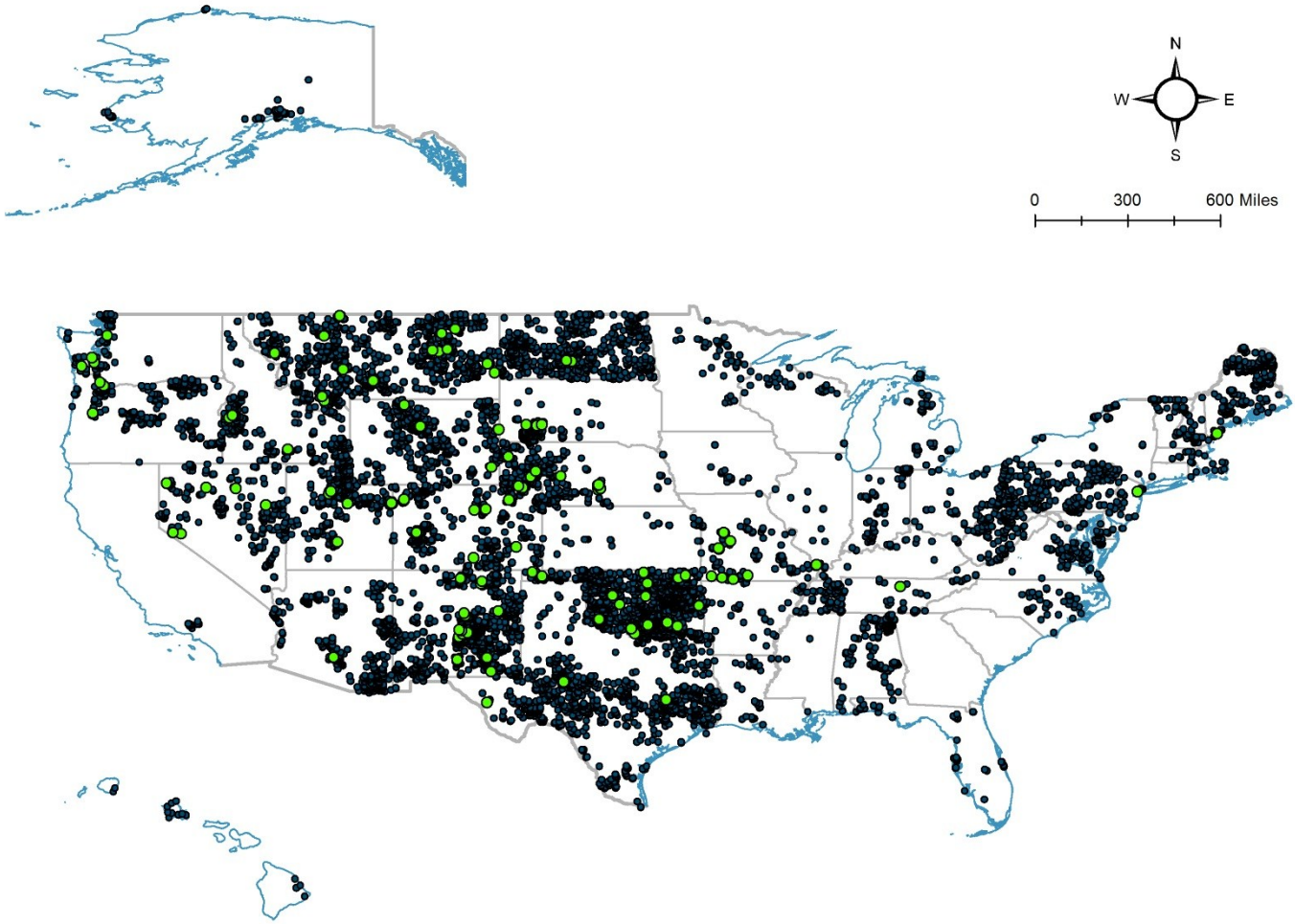


Figure 2: Tularemia surveillance in wildlife, 2005-2012. Blue symbols represent an *F. tularensis* negative sample; green symbols represent an *F. tularensis* positive sample.

NWDP Surveillance Projects

Leptospirosis

Leptospirosis is a bacterial infection caused by one of many *Leptospira* spp. serovars. Most mammals, including humans, can become infected. Humans typically become infected after exposure to water contaminated with animal urine that comes in contact with the skin, eyes, or with mucous membranes. Infections can range from asymptomatic to serious illness, involving kidney damage, meningitis, liver failure, etc. Infection in swine can lead to abortions or stillbirths.

The bacterial disease has been reported in a few feral swine in the United States, but little is known about the geographic distribution or apparent prevalence of leptospirosis in feral swine or other wildlife species throughout the country. Consequently, the NWDP utilized a subset of the feral swine samples stored in its National Feral Swine Serum Archive to screen approximately 2,000 serum samples with the microagglutination test at CSU. Samples were screened for exposure to 6 serovars (*Hardjo, Icterohemorrhagiae, Canicola, Grippotyphosa, Pomona*, and *Bratislava*) common in domestic animals or humans. Serovar *Hardjo* is of importance to the cattle industry and can also infect humans. *Icterohemorrhagiae* is typically found in rats. *Canicola* and *Grippotyphosa* are most commonly found in dogs and *Pomona* and *Bratislava* are most common in domestic pigs.

A sample was identified as leptospirosis positive if a titer was identified for any of the serovars; there is a considerable amount of cross-reaction between serovars making it difficult to be sure that a positive result for a particular serovar was not the result of a cross reaction with a different serovar.

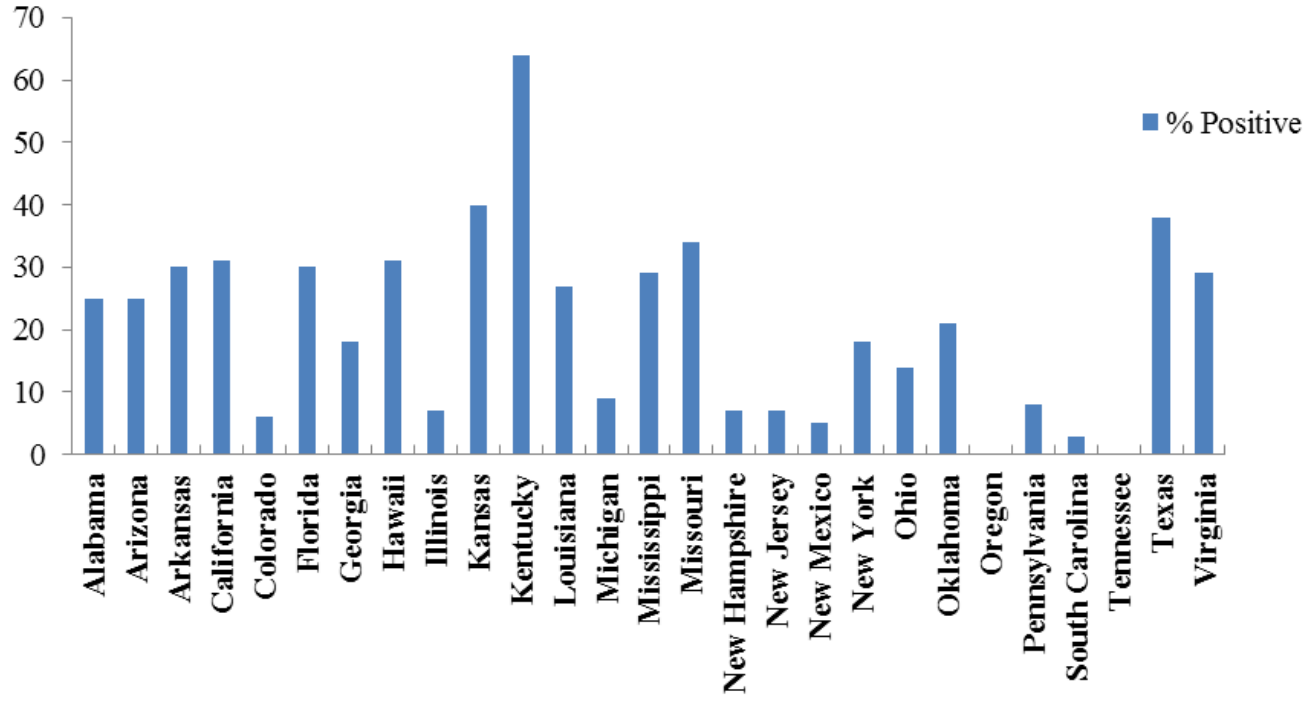


Figure 1: Percent Leptospira spp. seropositive feral swine, by state, for Fiscal Year 2012.

NWDP Surveillance Projects

Leptospirosis, continued

Figure 2: Feral swine sounder in urban setting.

Due to the relatively high apparent prevalence of leptospirosis in feral swine, the NWDP expanded surveillance to include testing of samples from raccoons and coyotes. Also, as an extension of the original project, feral swine kidney samples are being collected from counties previously identified as positive in the first phase of the project. The kidneys are being analyzed using a real-time reverse-transcription polymerase chain reaction assay to determine whether active shedding of *Leptospira* is occurring; the assay does not distinguish between serovars of leptospirosis. In addition, serum samples collected from feral swine in counties of states that have not previously been tested for antibodies to leptospirosis also will be analyzed.

During Fiscal Year 2012, 473 samples were collected and of these, diagnostics have been performed on 255 thus far. The samples are comprised of 76 coyotes, 74 feral swine, and 161 raccoons. Table 1 depicts the serological results by species. The results of the kidney samples are pending.

Table 1: Serology results for Leptospira spp. surveillance, by species.

Species	total samples collected	# of positives	# of negatives	# pending results
Feral Swine	213	32	42	139
Coyotes	83	19	57	7
Raccoons	177	63	98	16

NWDP Surveillance Projects

Neospora caninum

Figure 1: *Neospora caninum* can cause abortion in pregnant cattle.

In Fiscal Year 2011, the NWDP initiated a retrospective surveillance project to determine wildlife exposure to *Neospora caninum*. This project utilizes Nobuto blood filter strips from the Plague and Tularemia Archive, as well as serum samples stored in the Feral Swine Serum Archive. The specific objectives of this project are to determine if coyotes and feral swine are being exposed to the parasite, and to develop insights on the potential links between zoonotic transmission dynamics and livestock.

Neospora caninum is an intracellular, apicomplexan parasite that is similar in many ways to *Toxoplasma gondii*. Both are coccidian parasites, and both produce environmentally resistant, infective oocysts that are passed through the digestive system of definitive hosts as part of a complex reproductive cycle; however, while the definitive hosts for *T. gondii* are felids, the only definitive hosts for *N. caninum* that have been identified to date are canids, including coyotes, domestic dogs, wolves, and dingos. Examining *N. caninum* exposure in coyotes, which are likely a key definitive wildlife host in western states, will important information on the ecology of this parasite. Previous research has indicated a 10-11% exposure rates in limited numbers of coyote samples. To date, there is no information on *N. caninum* exposure in populations of feral swine in the United States, or if they have similar rates of exposure as cattle and sheep. In Europe, an 18.1% exposure rate in wild boar has been reported.

The primary interest in this organism stems from the isolation of *N. caninum* in cows, where it is the most frequently diagnosed cause of abortion in both dairy and beef cattle. Transmission in cattle is primarily vertical, with positive females passing the infection to calves. Epidemic outbreaks have been documented where > 50% of dairy cows in a herd abort within several weeks of each other, but losses in sporadic or endemically infected herds may be much lower because of an unpredictable recrudescence rate in infected cows. Infected adult cattle generally exhibit no clinical signs.

Limited information exists on economic losses associated with *N. caninum*, but one study estimated that the loss in California alone approached $35 million per year and a study in Texas estimated losses between $15 and $24 million per year. Economic losses stem from reproductive problems and abortion, loss of milk yield, reduced growth, and decreased feeding efficiency. In addition to cattle, *N. caninum* can cause clinical disease in dogs, as well as in sheep and goats, although the epidemiologic importance in these species remains unknown.

The NWDP surveillance project focused on overlapping populations of coyotes and feral swine (i.e., Texas, Oklahoma, and New Mexico) in order to capture disease dynamics in both a definitive host and an incidental host. Overall, 18% of coyotes (95% CL =14.2 - 21.8) and 15.8% of feral swine (95% CL = 12.5-19.2) had been exposed to the parasite. In addition, a subset of feral swine samples from Hawaii revealed exposure to the parasite, where the only definitive host present is the domestic dog.

Cooperative Activities

Newcastle Disease

Figure 1: Domestic poultry operations can suffer high mortality rates during a Newcastle Disease outbreak.

Newcastle disease (NDV) is a contagious viral disease affecting birds. Understanding this disease is crucial to the poultry industry because it causes illness, death and reduced egg production resulting in severe economic losses. In wild birds, the effect appears to vary depending on the species of bird and the virulence of the particular NDV strain. More than 230 species of birds have been found to be susceptible to natural or experimental infections with avian paramyxoviruses. Mortality due to NDV infection have been reported in cormorants, white pelicans, ring-billed gulls and California gulls.

Newcastle disease is caused by infection with an RNA virus within the avian paramyxovirus-1 group. Virulence of NDV varies with the strain and is measured by its ability to cause disease in chickens. Velogenic, or highly virulent Newcastle disease virus, are the most virulent strains and cause acute, lethal infection in chickens that can result in 100% mortality. These strains produce hemorrhagic lesions in the digestive tract. While rare in the United States, these viruses have been introduced on occasion, through illegal trafficking of exotic bird species. Neurotropic velogenic Newcastle disease is another virulent form of NDV that is generally lethal and affects respiratory and neurologic tissues. Morbidity is usually very

Cooperative Activities

Newcastle Disease, continued

high, but mortality is much lower than velogenic NDV. Mesogenic strains of NDV cause neurologic signs, but mortality is low. Lentogenic strains cause mild or unapparent respiratory infections in chickens, although some strains cause asymptomatic-enteric infections without visible disease.

While a national surveillance program for NDV is still under development , the NWDP encourages WS' wildlife disease biologists to investigate all morbidity/mortality events. The NDV is usually suspected when mortality rates are high and species such as cormorants, gulls, or pelicans are detected in regions where outbreaks frequently occur, such as the upper Midwest or northeast regions. For smaller scale mortality events (<500 birds), wildlife disease biologists are encouraged to submit samples to their state diagnostic laboratory and/or the NWHC in Madison, Wisconsin. For larger scale mortality events (>500 birds), wildlife disease biologists are advised to submit samples directly to the NVSL. The NWDP also collaborates with the Southeast Poultry Research Laboratory (SEPRL) in Athens, Georgia on developing a wild-bird specific assay for detecting NDV. Once NDV has been confirmed at another laboratory, SEPRL requests additional samples to validate the assay. In most cases, individual cloacal and oropharyngeal samples are collected and combined in a single 3 mL vial of brain heart infusion broth. Depending on where the samples are shipped, the entire carcass or specific organs may be submitted for testing. Results are reported back to the local office.

In addition to morbidity and mortality event investigations, the NWDP also collaborates on specific NDV research projects. During Fiscal Year 2012, the wildlife disease biologist from Minnesota collaborated with the NWHC to collect samples for a project to learn more about NDV in cormorants and gulls. Wildlife disease biologists from Michigan, Massachusetts, Rhode Island, Indiana, New York and New Jersey also collected samples as part of a mute swan surveillance project aimed at learning more about diseases and pathogens carried by mute swans (see Diseases in Mute Swans, page 28).

Figure 2: Areas where multiple species congregate can be sampled as part of disease surveillance activities.

Cooperative Activities

Avian Bornavirus

Borna disease is a neurological disease that primarily affects horses, but also sheep, goats, llamas, cattle, dogs, domestic cats, rabbits, deer, and lynx. It can cause fatal neurological and behavioral disorders in warm-blooded animals. Infection with Borna disease virus has been associated with some psychiatric disorders in humans. The virus is a non-segmented negative strand RNA virus (Order Mononegavirales; Family Bornaviridae). The name comes from the Saxon (German) city of Borna where the disease was common in the early 20th century, resulting in the deaths of more than 16,600 horses between 1896 and 1940. At least one confirmed natural host is the bicolored white-toothed shrew. Virus is shed in the shrew's urine. Other wildlife hosts have been proposed, but not confirmed. Borna disease virus appears to be globally distributed and, recently, it was determined that gene sequences related to the virus are incorporated into the human genome, as well as those of many other mammals and fish. This suggests a long association of Borna disease virus with vertebrate species.

Avian Bornavirus (ABV) is the etiological agent of proventricular dilation disease (PDD) in psittascine birds (parrots, macaws, conures). PDD is characterized by damage to the nerves of the enteric system. Food accumulates in the paralyzed proventriculus, eventually leading to death. Although PDD was first described in the United States in captive parrots and macaws during the late 1970's, no natural reservoir of ABV had been found in North America. In response to reports of two Canada geese on Prince Edward Island, Canada, displaying PDD-like neurological signs and lesions, researchers at the Texas A& M University, Department of Veterinary Pathology, speculated that waterfowl might be a possible reservoir.

Figure 1: Bornavirus infections have been identified in symptomatic Canada Geese.

Cooperative Activities

Avian Bornavirus, continued

Borna-infected birds are known to intermittently shed virus in the feces. A collaborative study was initiated when researchers contacted the NWDP to request access to cloacal/oropharyngeal swab samples from the Avian Tissue Archive (see Archive section, page 42). The objective was to retrospectively examine cloacal/oropharyngeal swabs from Canada geese for the presence of ABV, using reverse-transcription polymerase chain reaction. Also, brain tissue of apparently healthy, hunter-harvested Canada geese were assayed, and positive samples were isolated and sequenced.

In late Fiscal Year 2010, the Avian Tissue Archive staff supplied over 400 Canada goose samples representing 12 states and all four United States migratory flyways. In addition, NWDP wildlife disease biologists in New Jersey and Kansas submitted heads of apparently healthy, hunter-harvested or nuisance Canada geese and lesser snow geese for testing. At the Texas A&M Veterinary Diagnostic Laboratory, RNA was purified from swab samples, and cDNA was generated from brain tissues. Brain isolates were cultured in duck embryo fibroblasts and sequenced. To demonstrate that the results were not due to artifacts from possible laboratory contamination, the procedures were repeated at a second laboratory with no known history of Borna disease virus exposure.

Bornavirus sequences were detected in 12/409 swab samples (approximately 2.9%). Positive samples came from five states in three flyways (Atlantic, Mississippi and Pacific). Bornavirus was also detected in 13/25 (52%) of brain samples. Findings suggest that bornavirus might be quite common in apparently healthy Canada geese, but more sampling must be conducted to support this conclusion. The lower rate of detection in swab material is not unexpected, since studies of infected captive birds have shown that fecal shedding of detectable levels of the virus is intermittent.

Phylogenetically, the Canada goose brain isolates formed a tight cluster distinct from the psittacine ABV. In fact, the Canada goose isolates were more similar to the mammalian viruses than to avian bornavirus. They also align closely with a previously recovered sample from an encephalitic Canada goose. Similar analyses of brain tissue isolates from lesser snow geese in Kansas, mute swans in Michigan and several New England states, and ring-billed gulls in New York, have also been conducted. Positive samples have been found in all cases, with apparent prevalence in these studies ranges from 5-50%. The feeding habits of swans, geese and gulls are conducive to disease spread within flocks by the fecal-oral route, which has been identified as a likely mode of transmission. The results of this study suggest that Borna disease virus infection may be fairly common in apparently healthy waterfowl and gulls, although clinical infection appears to be rare, suggesting that they may serve as reservoirs. However, their ability to transmit the disease to other animals remains unknown. There is also some limited evidence of clinical ABV infection in raptors.

Recently, in July 2012, the NWDP Plague and Tularemia Archive supplied a number of dried blood Nobuto strips to Texas A & M. These samples were from small mammals associated with wetland habitats near areas where positive waterfowl have been found. These included raccoon, muskrat, nutria, beaver and mink. Results for these mammal samples were negative. The laboratory is now looking for antibodies to ABV using other tests. Future research will try to elucidate the host range among birds and small mammals, determine modes of transmission, and the frequency of clinical infections in wildlife.

Cooperative Activities

Disease in Mute Swans

Figure 1: Mute swans

Mute swans are considered an invasive species in many parts of the United States. They are prolific birds that are not typically afraid of humans, which can lead to large swan population densities in many urban areas. They cause significant ecological damage by eating submerged aquatic vegetation that serves as food for native waterfowl and habitat for fish and crabs, and their aggressive nature can prevent other birds from nesting, as well as threaten human safety. Some WS programs in the Great Lakes region received funding from the Great Lakes Restoration Initiative to lethally remove mute swans with the goal of minimizing ecological damage. The NWDP wildlife disease biologists in this region and in the northeast have been taking advantage of the swan removal by opportunistically collecting samples for disease surveillance.

The NWDP set up collaborative projects to look at specific diseases with Michigan State University, ARS, SCWDS, and the University of Texas Medical Branch. One of the primary diseases of concern is Newcastle disease virus (NDV), which can affect many domestic and wild birds. There are many die-offs that occur in the Great Lakes region and northeastern United States that are attributed to NDV. If mute swans are infected they may transmit the disease to other susceptible wild birds or poultry.

Other pathogens of concern include the *Schistosoma* parasites, one of which is the cause of swimmer's itch

Disease in Mute Swans, continued

in humans. Another parasite of concern is *Toxoplasma gondii*, a protozoan that infects most mammals, including humans, and results in a parasitic disease called toxoplasmosis. Samples were collected to evaluate their exposure to *T. gondii.*

Avian influenza is another disease of interest, especially in mute swans. In Europe, mute swans have been implicated in carrying H5N1. Serum samples were collected to evaluate exposure and combined cloacal/ oropharyngeal samples were collected to further identify the specific subtypes of avian influenza circulating in mute swans. Swan samples were also submitted for avian bornavirus testing, a disease previously thought to be found only in parrots and other pet birds.

Swans were lethally removed and samples were collected within 1 hour of death whenever possible. Serum samples were collected for influenza, NDV, and toxoplasmosis exposure. A fecal sample and the intestine were collected for parasite analysis. Combined cloacal and oropharyngeal sample were collected for avian influenza and NDV testing. The entire head was collected for avian bornavirus testing. Samples were sent to various laboratories including Michigan State University, ARS, SCWDS, and the University of Texas Medical Branch.

During Fiscal Year 2012, 397 mute swans were sampled. Samples were collected from Michigan (52%), New Jersey (25%), Rhode Island (13%), New York (3%), Massachusetts (3%), Wisconsin (2%) and Indiana (2%). The results that have been completed to date are summarized in Table 1. Testing that resulted in only a few positives was discontinued in FY12. Once testing is complete for all diseases, the results will be assimilated into a manuscript that will be submitted for publication.

Table 1: Number of mute swan samples screened, along with positive and negative results, for six pathogen groups.

Disease	FY11		FY12	
	Negative	Positive	Negative	Positive
Newcastle	140	308	217	153
Salmonella	455	3	Discontinued	
Parasites	254	83	331	13
Avian Influenza (serology)	35	149	189	155
Toxoplasmosis	213	41	203	6
Eastern Equine Encephalitis	24	2	Results pending	

Cooperative Activities

Canine Parvovirus

Canine parvovirus is a pathogen that can infect most canids. The disease likely emerged in domestic dogs in Europe during the 1970s and rapidly spread throughout the world in domestic and wild species. Scientists at the NWRC revealed that canine parvovirus entered western United States coyote populations during 1978 and serological data suggested it was enzootic in coyotes by 1980.

Figure 1: Coyotes are the primary samples being collected for canine parvovirus screening.

Canine parvovirus typically causes disease by infecting bone marrow, lymph nodes, spleen, and intestines in young animals that no longer have protection from maternal antibodies. The most common clinical sign is pronounced hemorrhagic enteritis (bloody diarrhea). Canids that become infected as adults often have no symptoms, but can remain infectious for up to 6 weeks. Although transmission through direct contact with infected animals is important, indirect contact with infected environments likely plays a more important role in the transmission and maintenance in a population. Canine parvovirus is extremely stable in the environment and transmission can occur when a susceptible animal has contact with feces, infected soil, or fomites.

While canine parvovirus can be controlled through environmental decontamination and vaccination of domestic animals and wild canids in captivity, implementation of such protocols in wild populations is not currently practical.

Although viral shedding has been documented in wild canids, most studies to date have focused on serological surveillance. To gain a better understanding of canine parvovirus in wild coyotes, the NWDP is initiating a collaborative surveillance effort with Cornell University to determine the distribution of canine parvovirus by identifying viral particles in tissues and feces.

To date the NWDP has collected samples from 799 animals representing 18 species of carnivores in 32 states and the territory of Puerto Rico. Diagnostic analysis has been completed on 355 animals with the results indicating that 13 states have positive wild carnivores.

Cooperative Activities

Nobuto Long-term Storage Study

The collection and transport of high quality samples is one of the primary challenges associated with wildlife disease surveillance. Samples must be able to retain useful information after being collected from field locations that are often remote, and under conditions that are less than ideal for maintaining biologically viability. Viable long-term storage of these hard to obtain samples is also a goal of many disease surveillance programs. A recent project has been initiated by the NWDP, in collaboration with the CDC to address the short- and long-term stability of plague antibodies on blood-soaked Nobuto strips under different environmental storage conditions.

The Nobuto strip is essentially a filter paper product that wildlife disease biologist and other field personnel use to collect blood samples for diagnostic analysis. A small amount of blood is collected on a Nobuto strip, which absorbs the sample and is allowed to air dry. The sample is then placed into a manila envelope. While this protocol has been used extensively in disease studies for many decades, a robust analysis that quantifies optimal storage conditions for blood-soaked Nobuto strips has yet to be completed.

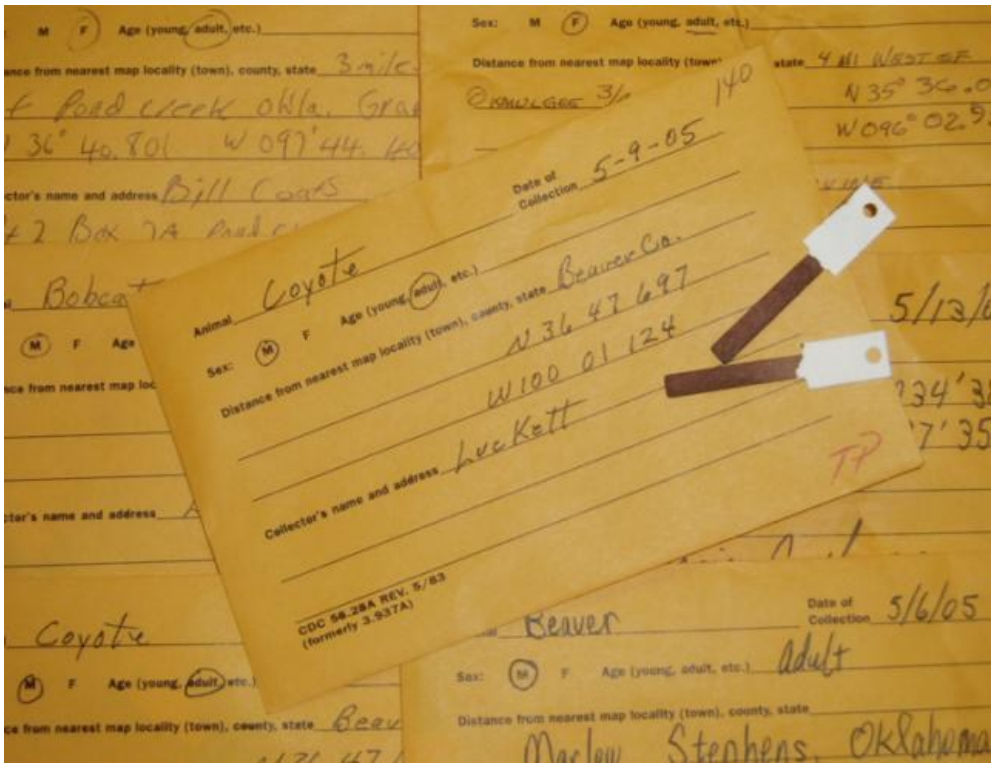


Figure 1: Blood-soaked Nobuto filter paper strips and data storage envelopes.

Data obtained through this study will provide robust information on the optimal storage conditions for Nobuto strip samples collected during the course of wildlife disease surveillance programs. Data will also help determine how best to maintain a long-term National Nobuto Archive, that will provide samples upon request from scientists.

Multiple Nobuto samples from known plague seropositive coyotes were collected and stored under ambient, 4^0 C, and - 20^0 C conditions. Humidity was monitored as well. Samples from all three temperature regimes were then tested periodically over time to determine if antibody titers changed over time. The samples are now being analyzed to determine the most effective environment to preserve sample integrity and to reduce protein degradation. Results are pending on this project.

Cooperative Activities

Comparison of Influenza Viruses in Wildlife and Humans

The NWDP is collaborating with ASU, The Mayo Clinic, and CSU to determine relatedness of influenza A viruses circulating in wildlife and humans in the Southwestern United States. The project, supported in part by a 2011 ASU-Mayo Clinic Seed Grant, is exploring the potential of using the health of wild animals as sentinels for zoonotic diseases in humans.

Figure 1: An example from previous research of how the relatedness of viruses can be viewed with the ZooPhy tool created by Dr. Scotch,.

This study uses samples collected during wild bird surveillance activities and stored in the Avian Tissue Archive which was established in 2006 (see page 42). Genetic material from influenza A matrix positive samples are being harvested by CSU personnel using virus isolation and lysis techniques. Once harvested, extracted RNA is sent to Dr. Matthew Scotch, the project's lead researcher based at ASU. Dr. Scotch performs reverse-transcription polymerase chain reaction to amplify the hemagglutinin (HA) segment of the genome. Cloning is then conducted using plasmid vectors transformed into competent E-coli cells. The plasmids are incubated overnight, extracted, and sequenced. The sequences are then assembled and the influenza subtype is determined by using the National Center for Biotechnology Information Basic Local Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi). The avian sequences will be compared with the HA sequences of human cases using a variety of bioinformatics techniques. One approach is to use Dr. Scotch's bioinformatics tool *ZooPhy* (zoophy.wikispaces.asu.edu), which models the dispersion of influenza in avian species and humans across the Southwest United States.

Thus far, 228 matrix positive samples have been harvested. Of those samples, seven viable viruses have been identified and genetic material sent to Arizona for genetic sequencing. Currently, the seven HA sequences have been completed and seven of these have been published in GenBank. The avian subtypes are: H8N4 (1), H4N6 (2), H11N9 (1), H3N8 (1), H6N1 (1). One sample is currently being sequenced and preliminary results suggest it is H3N8. This work was invited to be presented in two Bioinformatics and Molecular Epidemiology conferences and a publication is in preparation.

Detecting Infected Animals

Human health is inexorably linked with the health of our ecosystems and the animals that occupy them. Consequently, the USDA APHIS monitors the health and disease state of wild and domestic animal populations in the United States and abroad. In support of this mission, the NWDP has been collaborating with NWRC, Monell Chemical Senses Center, and CSU in developing novel approaches to detect and remove animals in the prodromal period of a disease state as a potential tool in managing disease spread.

It is known with certainty that some diseases are characterized by odor changes occurring in the infected individual. There is also convincing evidence that healthy individuals modify their social behaviors when exposed to the odors of infected conspecifics. It is this evolutionary communication system that this group of collaborators are attempting to exploit for development of new disease detection tools.

Collaborative studies conducted at Monell have conclusively demonstrated that immune responses triggered by treatment with rabies vaccine or lipopolysaccharide (a potent but relatively benign non-specific immune activator related to bacterial infection) result in body odor changes and that trained mice can learn to detect these odor changes. In fact, trained mice can discriminate between the LPS and rabies vaccine responses. Similarly, mice were trained to discriminate mallards experimentally infected with low pathogenic avian influenza from healthy animals.

Importantly, the infected animal need not be the source of odor. Because urine or feces provide the chemical cues used by trained mice to identify vaccinated or ill subjects, disease surveillance in wild animal populations may be possible without need to capture individuals. For example, chemical analyses of feces collected from healthy and infected mallards indicated that infection was associated with a marked increase of acetoin (3-hydroxy-2-butanone) in feces.

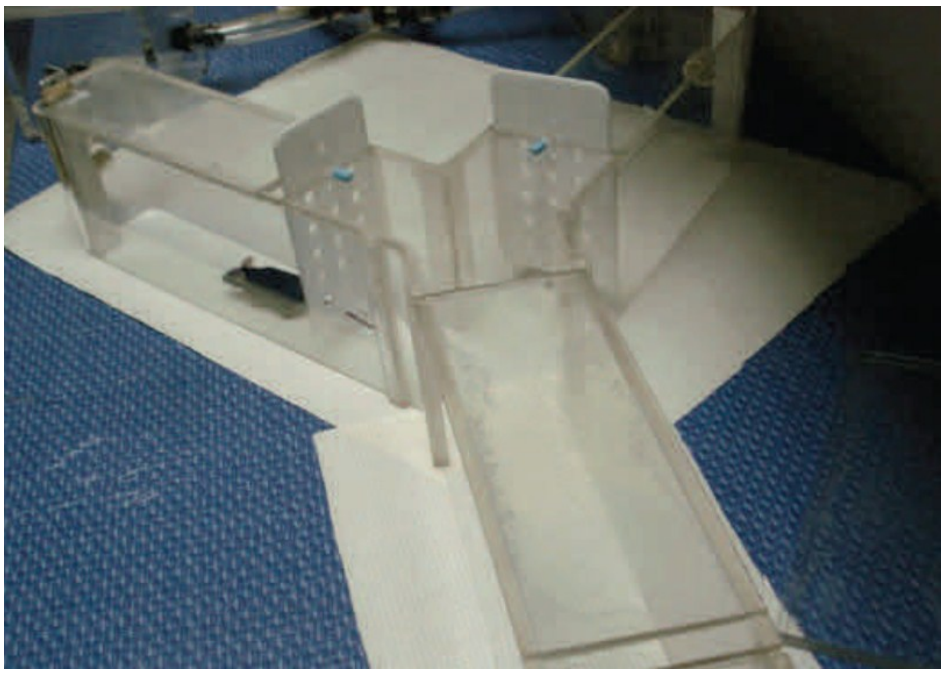


Figure 1: Mouse in a maze during odor experiments.

Thus, waterfowl health status may be evaluated noninvasively via monitoring of volatile fecal metabolites with trained biosensor animals or portable instrumentation. New laboratory studies will be initiated this year to determine if working animals can be trained to identify the infection status of waterfowl experimentally infected with various pathogens. If these trials are successful, additional studies will evaluate the ability of working animals in field sites to detect pathogens.

Cooperative Activities

Chronic wasting disease

Figure 1: Elk at fence line.

Chronic wasting disease (CWD) was discovered in Colorado and Wyoming in the early 1960's. CWD is a chronic, progressive, fatal, neurologic disease affecting mule deer, white-tailed deer, elk and moose. In the 1990's, CWD was discovered in captive cervid herds. Until about 2000, the disease in the wild was believed to be limited mostly to Colorado and Wyoming with limited incursion into surrounding states. In 2002, the disease was discovered in southern Wisconsin, far from its original focus. The finding of this disease in the Midwest initiated funding for intense surveillance throughout most of the United States. Intensive surveillance continued for about the next decade. In 2012 federal funding for CWD testing was significantly reduced, placing the burden of CWD surveillance in free ranging cervids largely on the states.

CWD in free ranging cervids has been detected in 17 states, mostly surrounding the original endemic areas of Colorado and Wyoming as well as southern Wisconsin and northern Illinois. In 2012 CWD was discovered in captive cervid herds in two additional states, Iowa and Pennsylvania. This brings the total number of states that have discovered CWD in captive herds to 13. Over the last 20 years, approximately 60 CWD positive captive cervid herds have been identified since the original South Dakota herd.

The NWDP provides assistance for CWD surveillance throughout the United States. In addition, the program has assisted with developing assays that can better detect infected animals and has also worked closely with operations in assisting VS with infected cervid farm depopulations. The program also provides subject matter expertise on CWD with lectures and sampling demonstrations, and is actively involved in collaborative research with CSU, NWRC, ARS, VS, and the Canadian Food Inspection Service.

One of the main problems in dealing with the transmissible spongiform encephalopathies, such as scrapie, CWD, and mad cow disease, is the lack of a live-animal test that would allow for early detection of the disease. Researchers in Norway and the United Kingdom discovered that there was a band of relatively accessible rectal lymphoid tissue that could be biopsied in live sheep. In order to generate the number of samples needed to validate the rectal biopsy procedure for diagnosis of CWD in white- tailed deer, the USDA, Canadian Food Inspection Agency, and CSU are collaborating to examine infected herds in Canada and the United States. The program hosted an international meeting to evaluate our data on the use of the rectal biopsy in white-tailed deer. This meeting resulted in the publication of validation results for the use of the rectal biopsy in white-tailed deer.

Cooperative Activities

Bovine Tuberculosis

Figure 1: Deer and calf interacting near feed.

Bovine tuberculosis (TB) is a chronic bacterial disease (primarily of cattle) caused by the microorganism *Mycobacterium bovis*. The disease can also infect other species, including humans and wildlife. Bovine TB is most often transmitted to humans by inhalation of aerosolized respiratory tract bacteria, ingestion of unpasteurized milk, and inoculation by contaminated instruments (such as knives). The disease can be spread from livestock to wildlife or wildlife to livestock via the fecal-oral route, ingestion of contaminated food, or though the respiratory tract. The APHIS Bovine TB Eradication Program has reduced TB in United States cattle; however, spillover into wildlife may maintain the microorganism in the environment and function as a source of re-infection for livestock.

The first documented cases of bovine TB in North American wildlife included white-tailed deer in 1933, 1937, and 1961. All of those cases were in the same area of New York. TB was subsequently found in free-ranging white-tailed deer in northern Michigan in 1975. A second occurrence from the same area of Michigan in 1994 drew attention to the possibility of wildlife being a reservoir host for *M. bovis*. Since 1995, *M. bovis* has been detected in several wildlife and feral species in North America, including white-tailed deer, mule deer, elk, bison, moose, raccoons, coyotes, opossums, feral cats, grey fox, black bears, feral swine, gray wolves, red fox, and bobcat, with varying degrees of infection.

The NWDP provides support for wildlife testing in states that have discovered *M. bovis* in livestock. Numerous wildlife disease biologists have been deployed to provide assistance in wildlife TB testing in Minnesota and Michigan, where spillover into white-tailed deer has occurred. While deer in MI serve as a reservoir, the situation in MN appears to be spillover without the establishment; MN appears to have eliminated the disease in deer.

In collaboration with VS, the NWDP has developed English and Spanish versions of the "Guidelines for Surveillance of Bovine Tuberculosis in Wildlife," which is available in bound copy, as well as electronically. The program introduced these guidelines to the United States/Mexico Bi-national Tuberculosis Committee at the 2012 winter meeting in Nashville, Tennessee.

The program has provided a presentation entitled "*Mycobacterium bovis* in North American Wildlife" on CDC's Zoonotic Disease conference call. The program will continue to provide expertise and assistance nationally and internationally as long as funding is available.

International Cooperation

Wildlife Reservoirs and Emerging Diseases

Habitat fragmentation and anthropogenic landscape change have brought humans and domestic animals in contact with zoonotic diseases that were previously limited to wildlife populations. This, combined with the speed at which commodities and people move around the world, has increased the risk of new diseases being inadvertently brought into the United States. Our knowledge of many of those pathogens is currently very limited, and increased understanding would be beneficial, both domestically and internationally. In many cases, the animal reservoirs of dangerous zoonotic diseases are still unknown, which makes it difficult to determine what threat exists for disease establishment in the United States. Employing a proactive disease surveillance system of suspected reservoir hosts could greatly increase our understanding.

The NWDP, in collaboration with CSU and collaborators in Cambodia, is using wildlife sampling protocols to collect samples from multiple species in Southeast Asia. In many cases, the role of wildlife in perpetuating diseases of interest, such as Chikungunya virus, is unknown. While there is laboratory data demonstrating the ability of some animals to act as disease reservoirs for these potentially emerging pathogens, what role that actually play in the field is a subject of debate.

In order to better understand the role of wildlife as reservoirs of viral zoonotic disease that could potentially be introduced into the United States, colleagues from the NWDP and CSU traveled to Cambodia to sample multiple species in regions that have been the site of recent human Chikungunya virus outbreaks. Human infection rates were the highest seen in the past 35 years. More than 500 animals were sampled from mammals, reptiles, and amphibians. Samples were taken in multiple regions through the country. All samples will be screened for Chikungunya virus, but an attempt will be made to screen for other pathogens as well. Any active infections that are isolated from these samples will be characterized. Overall pathogen prevalence patterns in sample species will also be determined. This initial set of samples will help clarify what pathogens can be detected and help target species of interest for future, more in depth, studies. Having more complete information on the dynamics of these pathogens will help to inform when and where infection risk is greatest for human populations, and will determine the potential for introduction and establishment of these diseases in new regions and countries.

Figure 1: Zoonotic disease surveillance encompassed multiple species.

International Cooperation

Mongolia

The NWDP has been collaborating with colleagues in the Mongolia Academy of Sciences and CSU for three years on a monitoring project for highly pathogenic avian influenza (HPAI) in Mongolian wild birds. Samples have been collected in Mongolia where a number of migratory bird die-offs have occurred due to the virus.

Figure 1: Sampling swans at Khar Us Lake, western Mongolia (photo by Nyambayar Batbayar).

The bar-headed goose was the principle species involved in the well-known HPAI H5N1 wild bird outbreak at Qinghai Lake in North-central China in 2005, as well as a die-off at Erkhel Lake in Mongolia in 2006. Both sites are in the Central Asian flyway. The Qinghai Lake event was the first documented large scale die-off of wild birds due to HPAI H5N1. Thousands of bar-headed geese died at Qinghai Lake, about 10% of the entire population, which is now estimated at about 36,000 birds.

Mongolia presents unique advantages for the study of avian influenza in wild birds. About 70% of the bar-headed goose population breed on Mongolia's vast grasslands and wetlands each year. The natural ecology of avian influenza in wild birds can be studied in isolation from interactions with domestic fowl because there are almost no commercial domestic poultry or backyard poultry in Mongolia. In addition, large numbers of other waterfowl and shorebirds breed or pass through Mongolia during annual seasonal migrations along the Central Asian flyway. In the Palearctic zone, the East Asian-Australian flyway bridges the gap between the Central Asian flyway and the North American Pacific flyway. Thus, through flyway interactions, there is potential for HPAI H5N1 to cross into the North American Pacific flyway via wild bird movements.

From Fiscal Year 2009 to 2011, biologists with the Wildlife Science Conservation Center and the Institute of Biology at the Mongolian Academy of Sciences, have collected approximately 2,000 samples from birds and small mammals, including tracheal swabs, fecal samples, serum, and tissues. The samples are packed in nitrogen vapor shippers and transported from Mongolia to CSU, where they are tested for influenza virus. Samples are assayed by real-time reverse-transcription polymerase chain reaction in a Biosafety Level 3 laboratory at CSU. Positive samples are further characterized by virus isolation methods and genetic sequencing.

International Activities and Collaboration

Figure 1: Participants in the 2012 Wildlife-Borne Disease Symposium.

The transmission of wildlife diseases is a global concern. Wildlife species are active in almost every environment, and often there is the potential for them to interact with domestic animals. Seasonal migrations and natural dispersals of many species can spread pathogens significant distances; migrations can range for thousands of kilometers, crossing many countries and covering multiple continents. These movements are only rudimentarily understood and impossible to control. Whether these species serve as potential bridge species to transmit diseases, or as hosts to enable pathogens to persist, is often unknown and potential problems are frequently not investigated. International collaborations increase our understanding of potential threats and provide knowledge of emerging zoonotic diseases. While sharing disease monitoring networks enables neighboring countries to be better prepared and coordinate control tactics, collaborative workshops and scientific exchanges provide valuable experiences for our scientists to work with foreign diseases before they enter the United States. We have contributed to improving the capacity to conduct surveillance for wildlife diseases in some countries. Working alongside foreign biologists not only facilitates exchange of knowledge that is mutually beneficial, but also increases the trust and familiarity among agencies that is essential when preparing for, or responding to, an emerging issue.

The NWDP has several long-term activities addressing wildlife disease issues. Over the past several years, the program has conducted wildlife disease surveillance or training for persons from over 30 countries spread across Asia, Africa, and South America. The NWDP serves as a national associate on the Food and Agriculture Organization of the United Nation's Scientific Task Force on Wildlife Diseases.

The NWDP collaborated with Chinese Academy of Sciences and the Chinese State Forestry Administration to conduct the Wildlife-Borne Disease Symposium during the International Union of Biological Sciences General Assembly, Conference on Biological Sciences and Bio-industry in Suzhou, China. Subsequently, staff collaborated with Chinese Academy of Sciences, Institute of Zoology to present an overview of wildlife disease surveillance to the Chinese CDC. Training was also provided in the United States for a group of leaders within the Chinese State Forestry Administration who will be working with wildlife disease issues. The NWDP supported two workshops in Peru to train specialists in conducting wildlife disease surveillance, and collaborated with FDA on strategies to investigate possible wildlife reservoirs of *Salmonella* on papaya plantations in Mexico. Collaborations were developed with the Faculty of

International Activities and Collaboration, continued

Veterinary Medicine from the Autonomous University of Mexico State in Toluca. In addition, the NWDP continued collaboration with the Canadian Food Inspection agency on chronic wasting disease research. Staff worked with FAO and USDA International Services to develop a project to alleviate damage caused by Nehring's blind mole rat.

Collaborative projects with CSU and the Cambodian Wildlife Protection Office were implemented to identify diseases in bats and to identify potential wildlife reservoirs for Chikungunya viruses in Cambodia (see page 36). Collaborative efforts with CSU to assist graduate students in Indonesia working on projects investigating disease transmission in wildlife markets were finalized.

The NWDP collaborated with multiple partners to investigate the potential role of wild suidae in the spread of African swine fever (ASF) to domestic swine. Two activities addressed the potential role of European wild boar in the Ukraine. The NWDP collaborated with USDA FAS to improve capacity within Ukraine to conduct disease surveillance in wildlife. Specific objectives for this project include strengthening Ukraine's wildlife management and disease surveillance program, promoting effective and efficient use of samples collected from the field to broaden surveillance of animal diseases, and strengthening the existing response to emerging disease outbreaks in wildlife. The NWDP also gained experience in surveillance and control of ASF through this project. Another related project with Southern Research Institute is addressing capacity in Ukraine to collect and process samples from wild boars to test for ASF and classical swine fever. The NWDP developed and implemented a course on Wildlife Damage Management with an emphasis on addressing African swine fever for a Ukrainian delegation held in Fort Collins, Colorado and San Antonio, Texas. Another workshop was developed for training Ukrainian scientists on wildlife disease surveillance to be held in the Ukraine during 2013.

The NWDP also collaborated with multiple partners in Uganda and Kenya to improve understanding of ASF in native wildlife species. Collaborations with Makerere University and Swedish Agricultural University are applying a molecular ecological approach to understand the role of bushpigs in the epidemiology of ASF at the wildlife-livestock interface in Uganda. There is also a collaborative project in place with the US Army Medical Research Unit-Kenya, the Central Veterinary Laboratories Kabete, the International Livestock Research Institute, and others. This group is investigating ASF in desert warthog and wild pig populations in north-eastern region Kenya.

The NWDP plays a primary role implementing a Memorandum of Understanding (MOU) between the Chinese Academy of Sciences, Bureau of Life Sciences and Biotechnology, and WS to promote ongoing cooperation. The MOU targets several areas for desired collaboration on wildlife disease issues, such as identifying risks posed by wildlife disease that threaten agriculture or have zoonotic implications; developing a joint consortium to address emerging disease issues in wildlife populations; developing management approaches for mitigating zoonotic disease impacts; promoting global awareness of threats posed by emerging wildlife diseases; and helping organize activities that enhance the region's capacity to respond to emerging wildlife diseases. A NWRC Program Manager serves as the co-chair for the bilateral working group formed to provide guidance on joint activities. Thus far, the bilateral working group has held biannual regional conferences on wildlife diseases in Asia, collaborated on surveillance activities for wildlife-borne diseases, developed an Asia-Pacific Wildlife Disease Network, and provided training for the Chinese State Forestry Administration to conduct wildlife disease activities.

Emergency Management

Response to Emergencies

Figure 1: Emergency response trailer.

The key to effective emergency response is a strong network of people trained and prepared to respond when the need arises. Responding to emergencies is common for WS. On most days every state receives multiple calls to assist with a problem or resolve an issue. WS has a cadre of professionals for whom these emergencies are routine, though each often shows its own unique characteristics. Most responses occur at the local or state level. However, occasionally additional resources are required for larger emergencies of to meet specialized needs for skills or equipment. The NWDP coordinates national emergency response capacity within WS. Program staff works with ESF-11 Coordinators to maintain an awareness of available resources through WS and ensure that the procedures and protocols required for initiating and implementing a response are available. The NWDP also ensures a cadre of employees is trained and prepared to respond to disease outbreaks in wildlife that threaten agriculture or human health.

During Fiscal Year 2012, NWDP staff contributed to APHIS response capacity by continuing assistance on the ROSS Directory. Staff also developed an alternative ICS training paradigm that changed training requirements for WS employees, which was eventually a factor in APHIS reviewing its training protocols. Staff also coordinated with other APHIS Emergency Programs to develop and implement ESF-11 protocols and served as a liaison with ESF Emergency Response Regional Coordinators. The NWDP represented WS at the ESF National Meeting in Washington D.C., participated on the WS Contaminants Response Working Group to ensure communication among WS employees, and developed specialized criteria for responses to emergencies involving contaminants (e.g., oil spills) and other emergency activities. Staff also reviewed WS Emergency Communications Standard Operating Procedures and other WS emergency related documents.

All wildlife disease biologists within NWDP are regarded as WS initial responders for any APHIS Emergency Response, and as primary responders for disease outbreaks in wildlife. These biologists are expected to maintain awareness of emerging wildlife disease issues, merge existing knowledge with new technical advances, and adapt to changing surveillance activities. Wildlife disease biologists also are expected to be alert for emerging wildlife disease issues in their assigned state. Recognizing concerns and being prepared to respond often requires an awareness of activities in other states with similar wildlife

Response to Emergencies, continued

species or habitats that are prone to similar pathogen exposure. The NWDP applies multiple approaches to assist wildlife disease biologists to maintain necessary skills, abilities, and knowledge to perform well in these positions. Necropsy and foreign animal disease information is taught through formal courses. All wildlife disease biologist received training for these issues during Fiscal Year 2012 through a week-long training in Athens, Georgia. The NWDP maintained first responder readiness of 41 wildlife disease biologists by ensuring compliance with FOH medical and respirator fit-test requirements.

Figure 2: Responding to a Chronic Wasting disease outbreak.

NWDP maintains three mobile emergency response laboratories. These mobile units consist of a diesel pickup truck, laboratory trailer, and all-terrain vehicle. The laboratory trailer also comes equipped with a mounted generator, free standing generator, refrigerator, heater and air-conditioning unit, centrifuge, dissecting scope, optical microscope, autoclave, large animal walk -on scale, necropsy instruments, and various supplies and equipment for a variety of tissue collection procedures. These units can serve as clean rooms, necropsy labs, mobile command centers, and hunter-check stations. During Fiscal Year 2012, NWDP worked to upgrade an additional larger mobile laboratory for use during disease investigations at remote locations. The program also acquired thermal cameras, thermal rifle scopes, and night vision scopes to allow emergency responders to perform night operations safely and effectively.

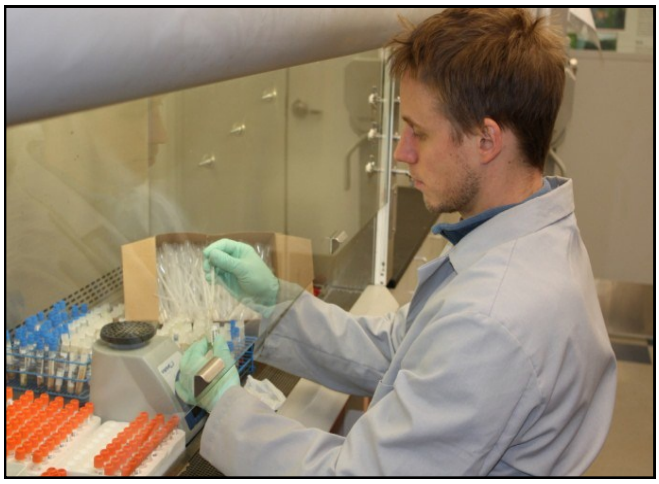
The NWDP provides coverage of an Emergency Hotline number (877-303-6363) to ensure rapid response to emergency requests. Calls to this number are forwarded to the assigned NWDP emergency contact person enabling response capacity 24 hours a day, every day.

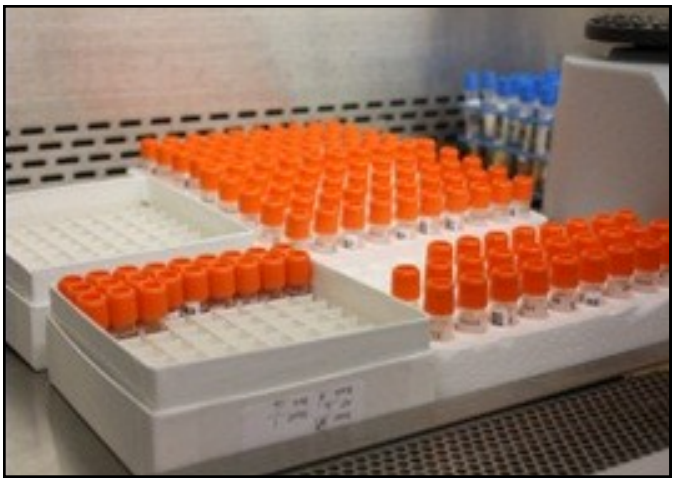
The NWDP successfully coordinated responses to 4 requests to address wildlife disease issues:

- Idaho Deer Hair Loss Syndrome response; consisted of mobilizing 4 wildlife disease biologists and emergency response trailer
- Missouri Chronic wasting disease surveillance, consisted of mobilizing 2 wildlife disease biologists
- Texas Tuberculosis surveillance; consisted of mobilizing one subject matter expert to provide technical expertise
- Missouri CDC Disease Surveillance Activity; consisted of mobilizing one subject matter expert to provide technical expertise and an emergency response trailer for a multi-agency disease surveillance operation

Archives

Sample Storage





Figures 1 and 2: Wildlife samples are held in multiple archives for long-term storage.

The NWDP was established in 2003 with a mission to provide a nationally coordinated system of surveillance and emergency response to diseases of concern in wildlife. In support of that mission, the wildlife tissue archives were established in 2006. The diverse contents of the archives are derived from the Interagency Highly Pathogenic Avian Influenza Early Detection System for Wild Birds, routine surveillance for diseases carried by feral swine, as well as monitoring for plague and tularemia in wildlife. The collection is unique in the quantity of samples, the diversity of species, the broad geographic range, and consistent sampling effort over extended periods of time.

The NWDP Wildlife Tissue Archives consists of three collections: The Avian Tissue Archive is cooperatively administered with CSU's Veterinary Diagnostic Laboratory, which houses the collection on their campus in Fort Collins, Colorado. The archive now consists of approximately 283,000 wild bird swab samples collected for highly pathogenic avian influenza surveillance in the United States from 2006-2011. Samples represent 260 wild bird species from all 50 states and several US territories. Over 1,900 samples were loaned to six universities in Fiscal Year 2012 for retrospective disease studies.

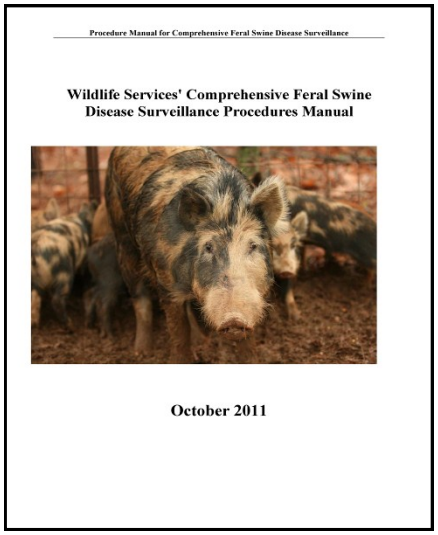
The Feral Swine Serum Archive consists of serum samples from feral swine collected in 36 states. In Fiscal Year 2012, samples from 2,894 feral swine were added to the archive. The samples were used to monitor 11 diseases carried by feral swine. The feral swine serum archive now contains serum samples from approximately 16,000 feral swine.

Over the years, 53,336 samples have been submitted to the Plague and Tularemia Archive for disease testing. While many of these samples were used in their entirety for disease testing, the archive currently houses 23,456 whole blood Nobuto filter strips from nearly 100 different species. The most frequently sampled species are coyotes, beaver, raccoons, skunks, and feral swine. There were 4,008 samples from 40 states added to the archive in Fiscal Year 2012.

The NWDP considers sample requests for research projects, retrospective disease surveillance, or diagnostic assay development, on an individual basis.

Procedural

Surveillance Manuals

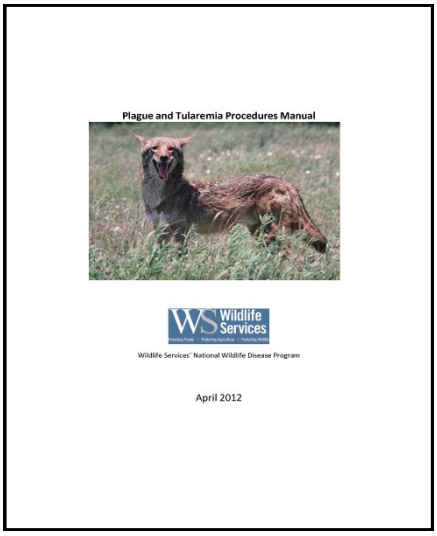

Procedure Manual for Comprehensive Feral Swine Disease Surveillance

Wildlife Services' Comprehensive Feral Swine Disease Surveillance Procedures Manual

October 2011

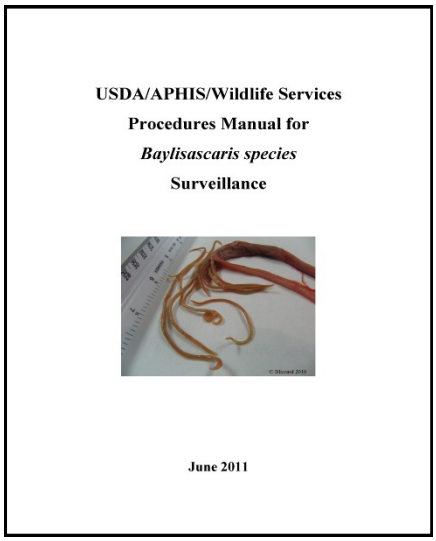

Toxoplasma gondii
Procedures Manual

October 2012

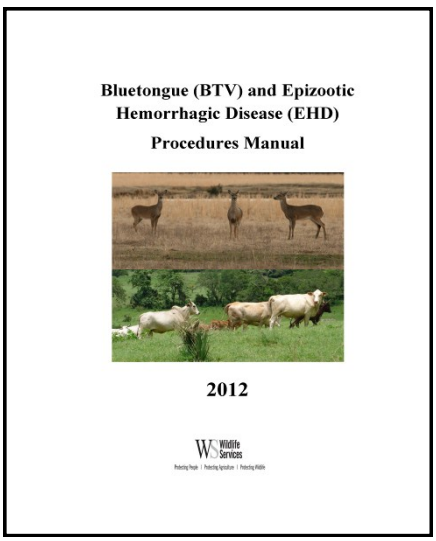

Plague and Tularemia Procedures Manual

Wildlife Services' National Wildlife Disease Program

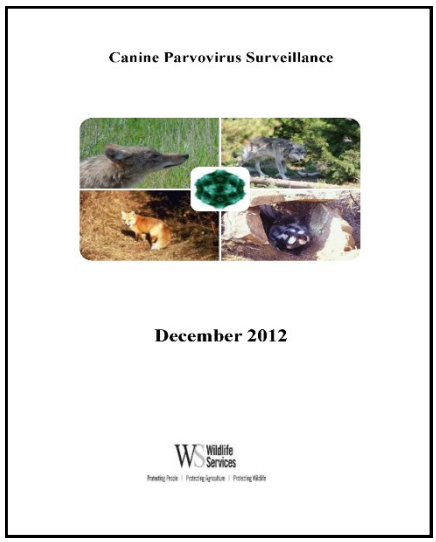
April 2012


USDA/APHIS/Wildlife Services
Procedures Manual for
Baylisascaris species
Surveillance

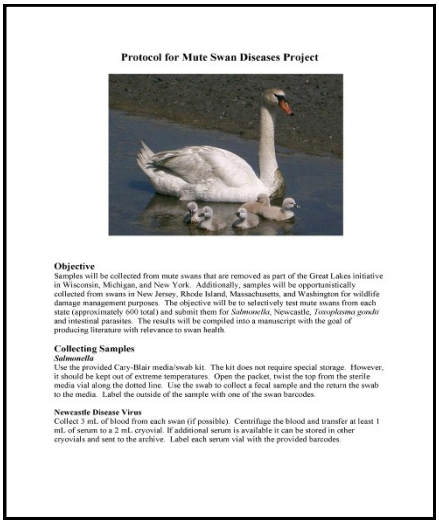
June 2011


Bluetongue (BTV) and Epizootic Hemorrhagic Disease (EHD)
Procedures Manual

2012


Canine Parvovirus Surveillance

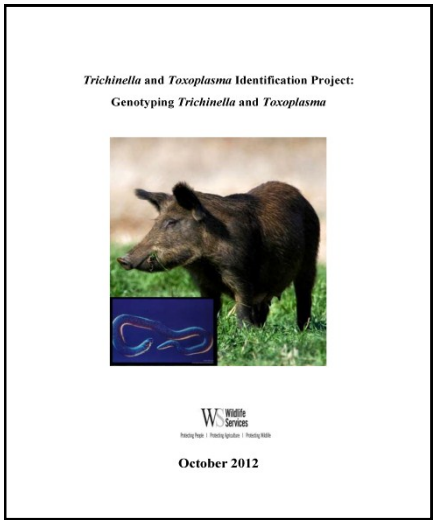
December 2012


Protocol for Mute Swan Diseases Project

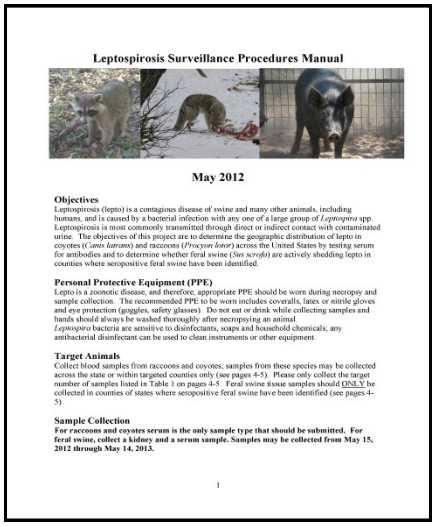
Objective
Samples will be collected from mute swans that are removed as part of the Great Lakes initiative in Wisconsin, Michigan, and New York. Additionally, samples will be opportunistically collected from swans in New Jersey, Rhode Island, Massachusetts, and Washington for wildlife damage management purposes. The objective will be to selectively test mute swans from each state (approximately 600 total) and submit them for *Salmonella*, Newcastle, *Toxoplasma gondii* and intestinal parasites. The results will be compiled into a manuscript with the goal of producing literature with relevance to swan health.

Collecting Samples
Salmonella
Use the provided Cary-Blair media/swab kit. The kit does not require special storage. However, it should be kept out of extreme temperatures. Open the packet, twist the top from the sterile media vial along the dotted line. Use the swab to collect a fecal sample and the return the swab to the media. Label the outside of the sample with one of the swan barcodes.

Newcastle Disease Virus
Collect 3 mL of blood from each swan (if possible). Centrifuge the blood and transfer at least 1 mL of serum to a 2 mL cryovial. If additional serum is available it can be stored in other cryovials and sent to the archive. Label each serum vial with the provided barcodes.


Trichinella and *Toxoplasma* Identification Project:
Genotyping *Trichinella* and *Toxoplasma*

October 2012


Leptospirosis Surveillance Procedures Manual

May 2012

Objectives
Leptospirosis (lepto) is a contagious disease of swine and many other animals, including humans, and is caused by a bacterial infection with any one of a large group of *Leptospira* spp. Leptospirosis is most commonly transmitted through direct or indirect contact with contaminated urine. The objectives of this project are to determine the geographic distribution of lepto in coyotes (*Canis latrans*) and raccoons (*Procyon lotor*) across the United States by testing serum for antibodies and to determine whether feral swine (*Sus scrofa*) are actively shedding lepto in counties where seropositive feral swine have been identified.

Personal Protective Equipment (PPE)
Lepto is a zoonotic disease, and therefore, appropriate PPE should be worn during necropsy and sample collection. The recommended PPE to be worn includes coveralls, latex or nitrile gloves and eye protection (goggles, safety glasses). Do not eat or drink while collecting samples and hands should always be washed thoroughly after necropsying an animal.
Leptospira bacteria are sensitive to disinfectants, soaps and household chemicals; any antibacterial disinfectant can be used to clean instruments or other equipment.

Target Animals
Collect blood samples from raccoons and coyotes; samples from these species may be collected across the state or within targeted counties only (see pages 4-5). Please only collect the target number of samples listed in Table 1 on pages 4-5. Feral swine tissue samples should ONLY be collected in counties of states where seropositive feral swine have been identified (see pages 4-5).

Sample Collection
For raccoons and coyotes serum is the only sample type that should be submitted. For feral swine, collect a kidney and a serum sample. Samples may be collected from May 15, 2012 through May 14, 2013.

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Peer Reviewed Manuscripts

2012 Publications

Besser, T.E., M.A. Highland, K. Baker, E.F. Cassirer, N.J. Anderson, J.M. Ramsey, K. Mansfield, D.L. Bruning, P. Wolff, J.B. Smith, and J.A. Jenks. 2012. Causes of Pneumonia Epizootics among Bighorn Sheep, Western United States, 2008–2010. Emerging Infectious Diseases 18:406-414.

Bevins, S. N., J. A. Baroch, D. L. Nolte, M. Zhang, and H. He. 2012. *Yersinia pestis*: examining wildlife plague surveillance in China and the USA. Integrative Zoology 7:99-109.

Bevins, S. N., S. Carver, E. E. Boydston, L. M. Lyren, M. Alldredge, K. A. Logan, S. P. D. Riley, R. N. Fisher, T. W. Vickers, W. Boyce, M. Salman, M. R. Lappin, K. R. Crooks, and S. Vandewoude. 2012. Three pathogens in sympatric populations of pumas, bobcats, and domestic cats: implications for infectious disease transmission. PloS One (Online: www. plosone. org) 7:e31403, doi:10. 1371/journal. pone0031403.

Brown V.L., Drake J.M., Stallknecht D.E., Brown J.D., Pedersen K., Rohani P. (2013) Dissecting a wildlife disease hotspot: the impact of multiple host species, environmental transmission and seasonality in migration, breeding and mortality. J R Soc Interface 10:20120804. DOI: 10.1098/rsif.2012.0804.

Carver, S, A.V. Scorza, S.N. Bevins, S.P.D. Riley, K.R. Crooks, S. VandeWoude and M.R. Lappin. 2012. Zoonotic parasites of bobcats around human landscapes. Journal of Clinical Microbiology 50:3080-3083.

Caudell, J. N., S. R. Stopak, and D P. C. Wolf. 2012. Lead-free, high-powered rifle bullets and their applicability in wildlife management. Human-Wildlife Interactions 6:105-111.

Clavijo, A., A. Nikooienejad, M. S. Esfahani, R. P. Metz, S. Schwartz, E. Atashpaz-Gargari, T. J. Deliberto, M. W. Lutman, K. Pedersen, L. R. Bazan, L. G. Koster, M. Jenkins-Moore, S. L. Swenson, M. Zhang, T. Beckham, C. D. Johnson, and M. Bounpheng. 2012. Identification and Analysis of the First 2009 Pandemic H1N1 Influenza Virus from U.S. Feral Swine. Zoonoses and Public Health: prepub.

DeLiberto, T.J., D.L. Nolte, and W. Clay. 2012. Integrative approaches to disease control: the value of international collaborations. Pages 175-180 in W. Karesh, ed. Compendium of the OIE Global Conference on Wildlife, Paris (France), 23-25 February 2011. World Organization of Animal Health, Paris.

Diel, D. G., P. J. Miller, P. C. Wolf, R. M. Mickley, A. R. Musante, D. C. Emanueli, K. J. Shively, K. Pedersen, and C. L. Afonso. 2012. Characterization of Newcastle disease viruses isolated from cormorant and gull species in the United States in 2010. Avian Diseases 56:128-133.

Farnsworth, M. L., R. S. Miller, K. Pedersen, M. W. Lutman, S. R. Swafford, P. D. Riggs, and C. T. Webb. 2012. Environmental and demographic determinants of avian influenza viruses in waterfowl across the contiguous United States. PloS One (Online: www. plosone. org) 7:e32729, doi:10. 1371/journal. pone0032729.

Fox A.D., Sinnett D., Baroch J.A., Stroud D.A., Kampp K., Egevang C., Boertmann D. 2012. The status of Canada Goose Branta canadensis subspecies in Greenland. Dansk Orn. Foren. Tidsskr. 106:87-92.

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2012 Publications Continued

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Hjulsager C.K., Breum S.Ø., Trebbien R., Handberg K.J., Therkildsen, Ole Roland , Madsen J.J., Thorup K., Baroch J.A., DeLiberto T.J., Larsen L.E., Jorgensen P.H. 2012. Surveillance for Avian Influenza Viruses in Wild Birds in Denmark and Greenland, 2007–10. Avian Diseases 56 (4): 992–998.

Jack, S. W.,. J. C. Cumbee, Jr., And K. C. Godwin. 2012. Serologic evidence of *Brucella* and pseudorabies in Mississippi feral swine. Human-Wildlife Interactions 6:89-93.

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Nemeth, N., A. Bosco-Lauth, P. Oesterle, D. Kohler, and R. Bowen. 2012. North American birds as potential amplifying hosts of Japanese encephalitis virus. American Journal of Tropical and Medical Hygiene 87:760-767.

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Pedersen, K., J. A. Baroch, D. L. Nolte, T. Gidlewski, and T. J. DeLiberto. 2012. The role of the National Wildlife Disease Program in wildlife disease surveillance and emergency response. Proceedings of the 14th Wildlife Damage Management Conference: 74-80.

Piaggio, A.J., Shriner, S.A., VanDalen, K.K., Franklin, A.B., Anderson, T.D., and Sergios-Orestis Kolokotronis. 2012. Molecular Surveillance of Low Pathogenic Avian Influenza Viruses in Wild Birds across the United States: Inferences from the Hemagglutinin Gene. PLoS ONE 7(12): e50834. doi:10.1371/journal.pone.0050834

Pedersen, K., S. N. Bevins, B. S. Schmit, M. W. Lutman, M. P. Milleson, C. T. Turnage, T. T. Bigelow, and T. J. Deliberto. 2012. Apparent prevalence of swine brucellosis in feral swine in the United States. Human-Wildlife Interactions 6:38-47.

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2012 Publications Continued

Sandfoss, M. R., C. S. Deperno, C. W. Betsill, M. B. Palamar, G. Erickson and S. Kennedy-Stoskopf. 2012. A serosurvey for Brucella suis, classical swine fever virus, procine circovirus type 2, and pseudorabies virus in feral swine (Sus scrofa) of eastern North Carolina. Journal of Wildlife Diseases 48:462-466.

Thomsen B.V., D.A. Schneider, K.I. O'Rourke, T. Gidlewski, J. McLane, R.W. Allen, A.A. McIsaac, G.B. Mitchell, D.P. Keane, T.R. Spraker, and A. Balachandran. 2012. Diagnostic accuracy of rectal mucosa biopsy testing for chronic wasting disease within white-tailed deer (*Odocoileus virginianus*) herds in North America: Effects of age, sex, polymorphism at PRNP codon 96, and disease progression. Journal of Veterinary Diagnostic Investigation 24:878-87.